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THE CULTURE, GENERAL PHYSIOLOGY, MORPHOLOGY, AND CLASSIFICATION OF THE NON-SULFUR PURPLE AND BROWN BACTERIA

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1. INTRODUCTION

"Eine Untersuchung des Stoffwechsels der heterotrophen Purpurbakterien erschien nicht nur deshalb, weil nähere Kenntnisse fehlten, als dankbare Aufgabe, sondern weil zu erwarten war, dass im Falle einer Kohlensäurereduktion durch diese Bakterien die Reaktion ganz anders verlaufen musste als bei den grünen Pflanzen und den Thiorhodaceen.   Und je mehr Varianten des Assimilationsvorganges wir kennen, um so eher können wir hoffen, Aufklarung über seinen Mechanismus zu erhalten."

H. Gaffron, (1), p. 2.

The studies on the green and purple sulfur bacteria have led to the demonstration that the normal metabolism of these organisms represents a truly photosynthetic process which differs in two major respects from that of the green plants.   Their biochemical activities have been interpreted as a photochemical carbon dioxide reduction with hydrogen which is ultimately derived, not from water as in green plants, but from hydrogen sulfide (2, 3).

In the first publication on this subject (2) I intimated that the sulfur-free purple bacteria, or *Athiorhodaceae* in the nomenclature of Molisch (4), which require organic substances for growth, might constitute a group of organisms in which the function of hydrogen sulfide as ultimate hydrogen donor for the photochemical carbon dioxide reduction might be here fulfilled by organic compounds

The tentative statement made in 1929 (2, p 168) "   . it is probable that the continued study of the metabolism of this group will give additional information with regard to the further possibilities of photosynthesis" was strongly supported by the experiments of H. Gaffron, published in 1933 and 1935 (1, 5)   In these it was shown beyond a doubt that a carbon dioxide reduction is actually accomplished by representatives of the *Athiorhodaceae*, and that this is fully dependent upon both illumination and the presence of organic substrates or molecular hydrogen

With the unambiguous demonstration by Foster (6) that the function of the organic substrate is indeed that of hydrogen donor for photosynthesis, the main outlines of the biochemical characteristics of this group were placed on a firm foundation.   Meanwhile Muller, in 1933, had shown (7) that *Thiorhodaceae*, too, can utilize simple organic compounds instead of hydrogen sulfide for photosynthesis.   Herewith the fundamental similarity in the metabolism of *Thio-* and *Athiorhodaceae* was established.

The various aspects of the problem of photosynthesis by bacteria have recently been reviewed in some detail (8)   It is, therefore, superfluous to deal here with this phase However, for studies on bacterial photosyntheses the use of representatives of the non-sulfur purple bacteria is, in many cases, preferable to that of *Thiorhodaceae*.   This is partly due to the fact that many of the former are far less sensitive to such external influences as the presence of small amounts of oxygen, and hence are more easily handled.   Also, the strains of bacteria belonging to this group have exhibited a capacity for attacking a greater variety of substances than have those sulfur purple bacteria which have been studied in this respect.   But different cultures of *Athiorhodaceae* display quite considerable differences in their biochemical potentialities   Although this has occasionally been recognized, a general survey of this aspect has never been published

During the studies on bacterial photosynthesis, carried out in the last fifteen years, a large number of strains of non-sulfur purple bacteria have been isolated in pure culture.

A more detailed investigation of the general morphology and physiology of this group has, therefore, been undertaken. It has led to the recognition of definite types which can be distinguished by both morphological and physiological criteria. As a consequence it has also become possible to attempt a more up-to-date system of classification of these organisms.

It is hoped that the publication of the results may make these bacteria more generally known to microbiologists, and thus lead to more intensive studies of the many fascinating problems which they still present.

2. DELIMITATION OF THE GROUP OF NON-SULFUR PURPLE BACTERIA

"Vergleicht man Molischs Ergebnisse mit denen der übrigen Beobachter, nament-lich Winogradskys, so kommt man bei vorurteilsloser Würdigung aller Umstände zu dem Schlusse, dass sich eine Scharfe Trennung zwischen schwefelhaltigen und schwefelfreien Purpurbakterien empfiehlt. Die typischen Vertreter beider Grup-pen gehören offenbar ganz verschiedenen ökologischen Kategorien an und weichen in wesentlichen Punkten ihres normalen Stoffwechsels weit voneinander ab. . . . Die hier betonte scharfe Gegenüberstellung kommt in Molischs Schrift nicht über-all klar zum Ausdruck."

Joh. Buder (9), p. 531.

It is advisable to outline what, in this treatise, will be understood by non-sulfur purple bacteria. In the past much confusion has resulted from the use of ambiguous names and inadequate definitions. This was largely the result of an incomplete understanding of the physiological activities of the various purple bacteria, and it should now be possible to avoid such difficulties by a careful appraisal of present-day information.

In 1907 Molisch (4) created the order *Rhodobacteria* for those organisms which he con-sidered "purple bacteria." They were characterized as bacteria containing a complex pig-ment system, made up of a green pigment, "bacteriochlorin," and one or more red pigments, "bacteriopurpurin." The order was subdivided into two families, the *Thiorhodaceae* and *Athiorhodaceae*. Diagnostically, these were distinguished by the occurrence of sulfur drop-lets in the cells of organisms belonging to the former family, whereas representatives of the latter always appear without sulfur droplets inside the cells.

Buder, in the report of his extensive studies on the purple bacteria (9), was the first to call special attention to the fact that these two groups of purple bacteria are primarily distinguishable by a fundamental difference in their metabolism. According to his views the *Thiorhodaceae* would represent organisms with an autotrophic, the *Athiorhodaceae* such with an heterotrophic mode of life; the former oxidizing hydrogen sulfide, the latter organic substances. This idea has been amply confirmed by the later investigations.

It should here be observed that the sulfur purple bacteria—as Winogradsky (10, 11) had already shown in 1887—contain sulfur globules only when growing in media containing sulfide. They use up this storage product during periods when they are exposed to a sul-fide-free environment. Since, then, the morphological characterization is not always strictly valid, subject as it is to environmental influences, it would seem better to use the intrinsic physiology as a basis for differentiation rather than its transitory morphological expression. On the other hand, it is well to remember that in nature one rarely ever encoun-ters typical *Thiorhodaceae* except under conditions where sulfide is present, so that sulfur-free specimens of this group may be considered as artifacts of the laboratory.

Although this last consideration would appear to make the morphological and physio-logical characterizations equivalent for practical purposes, another observation clearly favors Buder's approach. There have, namely, been found in nature small, rod-shaped purple bacteria which are physiologically speaking true *Thiorhodaceae* because they are capable of development in strictly mineral media with sulfide (3). Yet these organisms do not conform to the criteria set up by Molisch for this family because, even with an abundant sulfide supply, they never store sulfur droplets in their cells. The intermediate oxidation

product, sulfur, does not here become trapped *inside* the bacteria, but it accumulates in the external environment. Morphologically these microörganisms can therefore never be recognized as sulfur bacteria, while on the basis of Buder's physiological differentiation they should properly be grouped with the *Thiorhodaceae*. I should like to point out here that this consideration in an earlier publication (3) has given rise to a misunderstanding on the part of Pringsheim (12) who interpreted it as meaning that I opposed a subdivision of the purple bacteria into *Thio-* and *Athiorhodaceae*, whereas I merely intended to bring out the difficulties inherent in a purely morphological diagnosis and their ready elimination as a result of a physiological evaluation.

A more serious point is raised by the existence of types which appear to be intermediate between the two large physiological groups in the purple bacteria. Though Buder (9) failed to find experimental evidence in favor of such a concept, first introduced by Nadson (13), he felt compelled to consider it as a theoretical possibility, *and states:* "Sollten aber künftige Ergebnisse jene Angaben bestätigen, so wurden derartige Formen einen Übergang zwischen den beiden Extremen bilden. Wir hätten es dann mit mixotrophen oder fakultativ autotrophen Formen zu tun. So etwas wäre jedenfalls denkbar and hätte auch schon Analoga unter den Schizomyceten" (p. 536).

Now, as mentioned in the Introduction, it *has* been conclusively *demonstrated that all* the representatives of the typical *Thiorhodaceae* so far investigated in this respect are, in pure culture, not dependent upon the presence of sulfide, but can utilize organic substrates instead (7; see also 14, 15, 16). At first sight this might seem to erase the clear-cut physiological differentiation which Buder stressed. But it must be remembered that in natural environments the true sulfur purple bacteria do not come to the fore unless the medium contains sulfide, as has been clearly brought out by Winogradsky, who winds up his review with the claim: "Il n'y aurait, en conséquence, encore aucune raison valable de nier la nature essentiellement autotrophe des *Thiorhodaceae*" (17, p. 974).

Consequently it appears that a rigorous separation of the purple bacteria in Molisch's two groups of *Thio-* and *Athiorhodaceae* would be feasible provided: *a.* it is based upon physiological rather than morphological criteria, and *b.* due allowance is made for potentialities residing in the representatives of the first-mentioned group, but demonstrable only with pure cultures.

It is in this sense that the non-sulfur purple bacteria will here be treated. Even so, as will become apparent later, there exist cases in which it is rather difficult to assign to the organism in question a definitive position.

Whereas many bacteria of this group appear in cultures as a pink, violet, to deep-red growth, there also occur types which, though exhibiting essentially the same physiological characters, produce yellowish to dark-brown masses of organisms. It is probable that it is these bacteria which Utermöhl (18) has noted during his plankton investigations, and to which he refers as " . . . . eine neue, den Purpur- und Chlorobakterien vergleichbare Reihe farbstoffführender Bakterien . . . . , die ihrer braunen Färbung wegen wohl als Phaeobakterien bezeichnet werden können." But *Utermöhl was not* the first to find such "Phaeobacteria." Ewart, as early as 1897 (19), described such an organism under the name "*Streptococcus varians*"; and Molisch (4), in his descriptions of various (non-sulfur) purple bacteria, mentions a few examples of brown and reddish-brown pigmentation. Much later Gaffron (5), referring to his cultures of "brown bacteria," states: "Diese Bakterien sind sehr wahrscheinlich schon von anderen Forschern beobachtet worden. Eine Litteraturstelle ist mir nicht bekannt" (p. 307).

Organisms of this type are quite common in crude cultures of non-sulphur purple bacteria, as observed also by *Czurda and Maresch* (20). During the past fifteen years, numerous strains have been isolated and studied, morphologically as well as physiologically. In view of the fact that these bacteria contain a pigment system consisting of a functional bacteriochlorophyll accompanied by various yellow and red carotenoid pigments, there seems to be no valid reason for excluding them from the group of purple bacteria, even

though the cultures present colors which can certainly not be called "purple." They will, therefore, be included in the present treatise.

A discussion of the taxonomy and nomenclature of the group will be postponed till morphological and physiological characteristics of the several representatives have been described. Meanwhile, the organisms will be variously designated as "non-sulfur purple bacteria," "brown bacteria," or "*Athiorhodaceae*." Too much significance should not be attached to these names, however. They will merely serve to characterize the group of bacteria which forms the subject of this study.

3. THE CULTURE OF THE NON-SULFUR PURPLE AND BROWN BACTERIA

"Es war klar, dass trotzdem noch nicht alle Schwierigkeiten der Kulturmethodik beseitigt sein müssten. Ein Hinblick auf die Grünalgen genügt, um zu zeigen, wie trotz der gleichbleibenden Art und Weise des Kohlenstoffgewinnes doch noch eine so grosse Mannigfaltigkeit der ökologischen Ansprüche der Einzelformen vorkommen kann, dass nur ein Teil von ihnen heute einer sicheren Kultur zugänglich ist."

Czurda and Maresch, (20), p. 99.

I. Crude and enrichment cultures

a. Early methods; complex media. In the publications of Molisch (4), Buder (9), Ljubimenko (21), Schneider (22), Czurda and Maresch (20), etc., a number of general methods has been described for obtaining crude cultures of purple bacteria. The principle of all these methods is the exposure of a mixture of mud, surface water, and such ill-defined substrates as hard-boiled eggs, bones, preferably with meat, dead animals, such as worms, mussels, and other invertebrates, packets of leaves, etc., in a tall glass cylinder to light. After some time, usually of the order of magnitude of 1 to 2 weeks, purple bacteria begin to develop in such containers, and the crude cultures thus obtained are used for subsequent experiments.

An important improvement in methodology was introduced by Seliber in 1928 (23) and consists of a continuous illumination with artificial light. The time involved in getting the purple bacteria to develop until they form visible accumulations is thereby cut down to 3 or 4 days. In nearly all subsequent studies (2, 3, 5, 6, 7, 20) the continuous illumination procedure has been used to great advantage.

As a rule, the results obtainable with these methods are entirely satisfactory if one aims at securing crude cultures of any representative or mixture of representatives of this group of bacteria. It will, however, be obvious that the most diversified processes of microbial decomposition take place in such containers, in an utterly uncontrollable manner, and give rise to a more or less large variety of metabolic products. And these, in turn, may influence the types and numbers of purple bacteria which gradually make their appearance. By using these methods, it is thus left entirely to chance which species or combination of species will become sufficiently predominant so that their isolation can be achieved by the ordinary means of cultures on or in solid media.

The investigations of Molisch have made it abundantly clear that the group of *Athiorhodaceae* comprises a number of rather different types, including more or less spherical, rod-shaped, curved, and spirally-wound organisms, and Molisch

was the first to attempt a more detailed classification by creating a number of genera and species for the organisms studied by him (4). Since that time studies on the non-sulfur purple bacteria have been carried out with impure cultures or with pure cultures which had usually been acquired incidentally. The latter have been more or less adequately described and identified with one or another of Molisch's species. Only very recently has an attempt been made, by Czurda and Maresch (20), to isolate various representatives of this group with a view to studying their morphological and physiological characteristics, and thus to supplement the old descriptions and system of classification. But changes in the methods for obtaining crude cultures of *Athiorhodaceae* were not introduced.

b. Improved methods: theoretical considerations. During the past several years much information has been collected concerning the fundamental metabolic activities of the non-sulfur purple bacteria. This made it seem likely that methods could be worked out which would make it possible to secure cultures of different representatives at will. Once it is realized that these organisms are capable of photosynthetic activity in the light, and in the absence of oxygen, when provided with simple organic compounds, it also seems logical to expect that different organic substances will form the substrate *"par excellence"* for different types of *Athiorhodaceae*. With a more exact knowledge of their nutrient requirements it would become feasible to devise enrichment media for obtaining cultures of the desired type or group. Such methods would also tend to eliminate from the crude cultures many of the organisms which, in the complex and undefined media mentioned above, often comprise a large proportion of the microbial population, but which do not belong to the purple bacteria. The presence of such contaminants frequently makes it very difficult to proceed satisfactorily with the isolation of pure cultures of *Athiorhodaceae*.

Consequently several attempts have been made to apply these theoretical deductions to the development of more satisfactory and specific enrichment culture methods for the non-sulfur purple bacteria. Considering the photosynthetic activity as the outstanding physiological characteristic of all the purple bacteria, and the successful accomplishment of photosynthesis in the presence of a simple organic substance as the specific feature of the *Athiorhodaceae*, it was believed that a strictly mineral medium with an adequate supply of carbon dioxide, to which a single organic compound had been added, should suffice to ensure the development of the latter group of organisms from an appropriate inoculum, when exposed under anaerobic conditions to light. The results of these experiments, although not entirely satisfactory from the point of view of immediate success in the desired respect, are sufficiently instructive to justify a brief discussion. This is the more true because, particularly in the light of our present knowledge of the nutrient requirements of the *Athiorhodaceae*, they may well lead to the rapid development of more adequate methods.

c. Experimental results with different pure organic substances. For these experiments a standard mineral medium was used consisting of distilled or tap water with 1 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.2 g $MgSO_4$, 2 g $NaCl$ and 5 g $NaHCO_3$

per liter. This basal medium was then supplemented by the addition of a single organic substance in a final concentration of 0.15–0.2%, after which the reaction was adjusted to pH 7.0 with H_3PO_4. The organic compounds used were a number of individual members of the groups of simple alcohols, polyalcohols, fatty acids, hydroxy acids, dibasic acids, amines, and amino acids. A few cultures with complex nutrients, peptone and yeast extract, were always included. The various media were dispensed into glass stoppered bottles, inoculated with a small amount of surface water or mud-suspension, and the completely filled and stoppered bottles then incubated in a light cabinet (cf. (3)) at a temperature of 25° to 30°C under continuous illumination with a series of 25–40 watt electric bulbs.

Cultures of photosynthetic, red and brown colored bacteria develop fastest in the media with peptone and yeast extract. In 2 to 3 days one may usually count on an abundant growth of purple bacteria in the bottles. Next, the red organisms appear in media with ethanol, glycerol, mannitol, formate, acetate, succinate, malate, alanine. and asparagine; good cultures are regularly obtained in 4 to 5 days. The development of purple bacteria in media with higher fatty acids and other hydroxy acids is considerably slower; such compounds as isocaproic and heptylic acids often do not yield satisfactory cultures at all. Subsequent experiments have shown that this is due to too high an initial concentration of the fatty acids; the use of these substrates in concentrations of 0.05% or less yields excellent cultures in less than a week.

Microscopic examination of the various enrichment cultures revealed the presence of large numbers of easily distinguishable *Thiorhodaceae*. *Thiocystis* and *Chromatium* species occurred regularly, *Thiosarcina* not infrequently. In not too old cultures these bacteria present themselves stuffed with sulfur globules; a definite indication of the presence, and hence of the formation, of hydrogen sulfide in the media. The explanation of this fact is obvious; the enrichment media used allow of the development of sulfate-reducing bacteria (*Vibrio desulfuricans* and related species, cf. Baars (25)) which, by the dehydrogenation of the organic substrates with sulfate as the ultimate hydrogen acceptor, produce the sulfide. Transfers of such cultures to the same media under anaerobic conditions, but incubated in the dark, where the development of the purple bacteria is completely suppressed, have served to verify this statement; in all cases flourishing cultures of sulfate-reducing bacteria were obtained.

Thus it appeared that the enrichment cultures contained sulfate-reducing bacteria and, as a consequence of their development, *Thiorhodaceae*, in addition to one or more types of non-sulfur purple and brown bacteria. That members of the last-mentioned group were present was demonstrated by making use of their strongly phototactic behavior (4, 9, 24), a property not exhibited by the sulfate-reducing bacteria. Although, as all previous investigators have noticed, the small individual cells of *Athiorhodaceae* do not appear colored when examined with the microscope, the pigmentation is unmistakable in aggregations. It may at first seem arbitrary to conclude that such colored accumulations are composed of non-sulfur purple bacteria, based as it is on the observation of red

or brown clumps of organisms exhibiting phototaxis, and not containing sulfur droplets. A confusion with certain members of the red sulfur bacteria would be quite possible since the small *Thiorhodaceae*, described in 1931 as the "pseudomonas-type" (3), would behave in the same manner. However, the actual isolation of typical non-sulfur purple bacteria from all such cultures, and of a size and shape which agrees remarkably well with that of the cells in the phototactically induced aggregates, tends to justify the above conclusion.

d. Evaluation and applications. These results paved the way for a more appropriate method of enriching *Athiorhodaceae*, in which the simultaneous development of sulfate-reducing organisms and, in consequence, of sulfur purple bacteria could be eliminated. Since the growth of *Vibrio desulfuricans* and its relatives depends upon the presence of sulfate, the substitution of NH_4Cl and $MgCl_2$ for the sulfates in the standard mineral medium served the purpose; sulfate reduction did not proceed in such media, and *Thiorhodaceae* did not appear. It must be remembered that the media prepared with this modified mineral solution are essentially sulfur-free; and it soon appeared that the *Athiorhodaceae* develop only scantily upon repeated transfers. But if, after two or three subcultures, transfers are made into sulfate-containing solutions, the new cultures generally fail to show development of sulfate reducing bacteria and of purple sulfur bacteria. The first few subcultures have thus served the purpose of so modifying the initial ratio of *Athiorhodaceae* to sulfate reducers and sulfur purple bacteria that only the first group of microörganisms comes to a full development.

Inspection of such enrichment cultures containing different organic substances clearly indicated the fundamental correctness of the premises which had led to their use. The macroscopical appearance often revealed characteristic differences of color and mode of development; and this was fully supported by careful microscopical examination. Media with fumarate, malate, citrate, and ethanol invariably contained an abundance of red-colored *Spirillum* species; the more complex media with peptone and yeast extract usually showed the largest variety of types, with a preponderance of brown representatives of the *Athiorhodaceae*. The rod-shaped organisms, reminiscent of Molisch's genera *Rhodobacillus* and *Rhodovibrio* were encountered in the cultures with ethanol and with the higher fatty acids. It thus became evident that more or less specific enrichment cultures for different types of the non-sulfur purple and brown bacteria would be practicable. The systematic investigation of the physiological characteristics of the large number of pure cultures of this group, to be discussed later, has supported the evidence here obtained, and has furnished additional information with the aid of which it has now become possible to develop strikingly specific culture media for the enrichment of its various members.

A peculiar behavior of the enrichment cultures discussed above should be noted at this place. Successive transfers of such cultures with a single organic compound to homologous media often showed a progressively poorer development of *Athiorhodaceae*, both in the total number of organisms and of types. It seemed that in successive transfers the growth of these organisms came to depend more and more on the simultaneous propagation of non-photosynthetic

bacteria. Also, the differences in flora between the cultures in various media, originally so pronounced, gradually tended to disappear. Only in the media with peptone and yeast extract did the *Athiorhodaceae* continue to flourish without diminution in numbers or types, and from such cultures they could be isolated without difficulty.

The reason for this strange behavior has become understandable as a result of the later studies with pure cultures. Suffice it to say here that all the representatives of the photosynthetic non-sulfur bacteria appear to require, in addition to an organic hydrogen donor, one or more accessory growth factors. Thus, the simple mineral media with a single organic substance are inadequate for the development of *Athiorhodaceae*. It is probable that the necessary growth factors are furnished in the crude cultures by the simultaneous growth of other types of bacteria, including the *Thiorhodaceae*. My own experiments have provided adequate experimental support for this contention. And the curious observations of Czurda and Maresch (20) on the influence of colonies of *Chromatium* or *Thiocystis* species on the development of neighboring cells of *Athiorhodaceae* can be most satisfactorily accounted for on this basis. Also, it is well to bear in mind that several of the media used by no means exclude the simultaneous growth of non-photosynthetic organisms under anaerobic conditions. The existing information on the fermentation of polyalcohols, such as glycerol and mannitol, of lactate, fumarate, malate, citrate, etc., by various obligatory and facultative anaerobes (see, for example, 26, 27, 28, 29) makes this obvious. In addition, it is now common knowledge that microöorganisms, capable of developing in a medium devoid of the typical growth factors, do synthesize these substances themselves, and often in amounts far in excess of their own "needs."

With this in mind it is a simple matter to prevent the regression in vigor of the enrichment cultures for the non-sulfur purple bacteria. All that is necessary is the addition to the simple culture solutions of small amounts of material rich in growth factors. Yeast extract has proved to be entirely satisfactory; liquid autolysate, prepared according to Orla-Jensen (30), is used in amounts of 1 to 5 ml per liter of medium, while 0.1 g of dehydrated yeast extract usually suffices. Such enriched media have yielded dependable and reproducible results.

c. Elimination of green algae. Occasionally the first inoculum contains large numbers of green flagellates, such as *Euglena* and *Chlamydomonas* species, which may cause difficulties in obtaining good cultures of the purple bacteria. This is particularly the case if, due to the composition of the medium, the development of the latter is slow; it has often been observed with media in which tartrate and malonate constitute the sole organic compounds. Due to the rapid production of oxygen by the green forms the conditions in such cultures soon cease to be anaerobic, and in consequence a varied flora of more or less common aerobic, non-photosynthetic bacteria makes its appearance. Transfers at different stages of the development of such cultures fail to eliminate the flagellates. This can, however, be readily achieved by making use of the elegant method, first employed by Gaffron (5) for the same purpose, of using short infra-red illumination (800–1000 mμ) as a source of radiant energy. Since Engelmann's original

observations (31) it has become firmly established that the purple bacteria, in contrast to the green plants, are capable of utilizing infra-red radiation for photosynthesis. (e.g., French (32, 33)). But in most cases the use of special light filters in order to prevent the growth of algae is superfluous because the purple bacteria develop so rapidly that they soon overgrow the green organisms. In one or two transfers, particularly if made from young cultures, the algae will in general have been diluted out.

f. Effect of pH. Apart from using different organic substrates for the enrichment of various representatives of the *Athiorhodaceae*, experiments have also been conducted in which the same solution, but adjusted to different pH values, was employed. Such cultures with an acetate medium at pH 5.6, 6.2, 6.6, 7.2, 7.4, 7.7, 8.0, and 8.6 have not only yielded a variety of pure cultures of non-sulfur purple bacteria, but also shown that differences in the reaction of the medium certainly affect various representatives of this group in different ways. An initial pH below 6, together with an acetate concentration of 0.2%, is not conducive to the growth of any one type, nor is a pH much above 8. But in an

TABLE 1

Growth of Rhodospirillum rubrum and of a brown member of the Athiorhodaceae in media containing 0.2% Na-acetate, 3 days after inoculation

ORGANISM \ pH	6.1	6.4	6.6	6.8	7.0	7.6
R. rubrum	3*	6	4	3	2	1
Brown bacterium	0	1	1	2	5	2

* The numbers indicate relative densities of organisms.

acetate medium, originally at pH 6.2, spirilla become abundant in a few days, while at pH 7 and higher brown bacteria tend to be the more numerous. This is quite in line with the outcome of experiments with pure cultures of a strain of *Rhodospirillum rubrum* and of a "brown bacterium" in acetate media at different initial pH, as shown in Table 1. However, the reaction of a medium in which *Athiorhodaceae* develop does not, as a rule, remain constant. And since the use of different substrates provides a more convenient as well as a more certain way for obtaining enrichment cultures of various types, extensive further experiments on the effect of pH on the microflora of enrichment cultures have not been carried out.

g. Enrichment cultures with pasteurized inocula. During the progress of the work on enrichment cultures, observations were occasionally made which suggested the presence of sporeforming bacteria in the media. Several subcultures from such media have been made after subjecting the original culture to pasteurization for 5 to 10 minutes at 60° to 80°C. In none of the transfers did purple bacteria ever develop.

Also inoculations of those sterile media which had proved to be among the

most satisfactory for the enrichment of purple bacteria with pasteurized samples of mud and surface water have invariably failed to yield photosynthetic bacteria. The same experience has been reported to the writer by Dr. J. W. Foster (unpublished). The evidence to date therefore indicates that thermoresistant stages (endospores) are not produced by any of the organisms of this group.

h. Distribution; inocula. The previous pages have shown the simplest and, in many respects, most satisfactory approach to the methods for securing crude cultures of *Athiorhodaceae.* In a later section it will be pointed out how this method can be used to obtain strains of a definite species. Here, however, some remarks are in order concerning the best material to use for inoculation of the enrichment culture media, in connection with the distribution of the organisms in nature.

All investigators who, starting with Molisch (4), have attempted to grow crude cultures of non-sulfur purple bacteria in the laboratory, have remarked on the wide-spread occurrence of the organisms. But contrary to the oft-reported mass occurrence of the *Thiorhodaceae* (see, e.g., 34, 35), the *Athiorhodaceae* have not been encountered in macroscopically visible aggregations in nature. Buder (9) observes in this respect:

"So leicht nun die Anzucht und Kultur von Athiorhodaceen ist und so verbreitete Bürger sie nach Molischs und eigenen Erfahrungen in unseren Gewässern sind, so ist mir doch weder aus der Literatur noch aus eigener Anschauung ein ähnlich *auffallendes* Vorkommen in der freien Natur bekannt geworden wie bei den roten Schwefelbakterien. Immer waren es Aufgüsse und ähnliche Herrichtungen, in denen ihre Entwicklung eine solche Üppigkeit erreichte, dass sie sich schon dem blossen Auge durch ihre Farbe verrieten" (p. 535–536).

This passage holds equally good today. The red bacteria, so often occurring in large numbers in brines and salterns, or on salted fish or hides, have occasionally been considered as purple bacteria. But they are non-photosynthetic, and do not contain the typical pigment system of the *Thio-* and *Athiorhodaceae.* They cannot, therefore, be regarded as purple bacteria.

Nevertheless, it is difficult to collect samples of mud or surface water in which, by proper enrichment methods, one cannot demonstrate the presence of the brown or red non-sulfur purple bacteria. They often occur in considerable numbers, as shown by inoculating adequate media with progressive dilutions of the material. It may well be that they often accompany the *Thiorhodaceae* in their natural habitat, except in sulfur springs, in such numbers that they might be macroscopically visible. But the large, sulfur-containing bacteria are so much more conspicuous upon microscopic investigation; they are observably colored even as single individuals, and can thus so readily be made responsible for the coloration of the sample, that it appears superfluous to search it for other, small pigmented forms which can betray their colored nature only when viewed in masses.

The materials which seem to harbor the richest flora of *Athiorhodaceae* are, generally speaking, muds in which considerable microbial decomposition is going on, or the overlaying water. I have only on rare occasions obtained successful cultures from sand and soil samples; apparently the natural environment of the

organisms is an aqueous one. Though it is possible that rich soils actually may contain large enough numbers to yield positive results by using 1 to 5 g quantities as inoculum, it seems to me highly doubtful that a variety of the non-sulfur purple bacteria such as is readily procurable from stagnant ponds will ever be found there.

Since much of the present work is concerned with the group of *Athiorhodaceae* as a whole, it has been my purpose to acquire a varied collection of strains and types. With the aid of different media and inocula the collection thus built up comprises well over 150 strains, and the study of their characteristics seems to justify the conclusion that among them most, if not all, of the previously described representatives are found. The use of appropriate enrichment methods has made it possible to isolate practically all the different types from a single source, but various strains have been obtained from widely divergent inocula. It is, of course, quite likely that new types will be found in the future, perhaps from unusual environments, or by the application of different methods, and it is hoped that the general approach here presented may prove of assistance in further studies.

II. Pure cultures

When satisfactory enrichment cultures are available it is a relatively simple matter to proceed to the isolation of pure cultures. This might be surmised from the fact that Esmarch, as early as 1887 (36), succeeded in isolating the first representative of the group in pure culture by routine bacteriological methods. Also Molisch's account (4) of the manner in which he achieved pure cultures of *Athiorhodaceae* leaves one with the impression that the difficulties involved are not excessive.

The most important aspect is the harmful effect of oxygen on many of the purple bacteria, stressed by Molisch in the following passage: "*Die meisten Purpurbakterien wachsen nicht in ausgegossenen Platten, weil der leicht zugängliche Sauerstoff ihre Entwicklung hemmt oder ganz verhindert*, ferner ist ihr Wachstum gewöhnlich relativ sehr langsam, weshalb die Kolonien in deutlicher Form erst nach längerer Zeit erscheinen, und ausserdem tritt bei manchen, namentlich wenn sie noch relativ viel Sauerstoff empfangen, die rote Farbe erst später auf." (4, p. 11).

Since the enrichment cultures as well as pure culture studies have shown that all the members of the group, without exception, are capable of development under strictly anaerobic conditions, provided they are properly illuminated, the most certain *general* method is that of anaerobic incubation in a light cabinet, of solid media inoculated with material from enrichment cultures.

It should here be observed that not every anaerobic culture method is equally effective. In the absence of air the purple bacteria depend, for their development, on their photosynthetic activities. This will make it clear why Czurda and Maresch (20) encountered difficulties when using agar plates stacked in containers which were made oxygen-free by mixtures of pyrogallic acid and potassium hydroxide. The carbon dioxide tension in such an environment is decidedly too low for ensuring active photosynthesis on the part of the organisms.

The most satisfactory procedure is that of shake-cultures, using successive dilutions, in an agar medium, the composition of which can be varied at will. If such cultures are made in culture tubes, and the agar column after solidification is covered with a sterile, melted mixture of equal parts of paraffin and paraffin oil, they can be kept without drying out, and without becoming "aerobic" for many weeks, even months.

Concerning the composition of the medium the following may be said. A "universal" medium, i.e., one which has proved satisfactory for all strains studied, is a dilute yeast extract agar, containing 3 to 10 ml liquid yeast autolysate per 100 ml. The addition of a small amount of Na_2S (about 0.01% $Na_2S \cdot 9H_2O$), and of Na_2CO_3 or $NaHCO_3$ (0.2%) is recommended to provide strictly anaerobic conditions from the start, as well as a sufficient supply of CO_2. Although development of most of the purple bacteria can take place over a fairly wide pH range, adjustment of the reaction to pH 7 with a few drops of sterile phosphoric acid solution is preferable; at this pH the sulfide in the concentration used exerts no inhibitory effect, and yet can rapidly eliminate the last traces of oxygen.

For certain purposes the use of an agar medium of approximately the same composition as that of the enrichment culture medium may be desired. If so, it must be realized that in some media the purple bacteria, in crude cultures, occur principally as a secondary flora. Primary decompositions of the substrate under the influence of non-photosynthetic microörganisms give rise to changes in composition of the medium, and it is often the decomposition products, notably the fatty acids, which lead to the subsequent growth of purple bacteria. In general, acetate and butyrate are excellent substrates, with lactate and malate running a close second.

However, the concentration of the fatty acids should not be too high (0.2% as a maximum in the form of their Na salts), nor the pH of the medium below 7. Media of this composition, due to the complete utilization of the organic anion, tend to become extremely alkaline, however, and such cultures are therefore not as lasting as the ones in yeast extract. Whenever such "synthetic" agar media are used the addition of small amounts of yeast autolysate (1 to 5 ml per liter) is necessary to provide the required growth factors.

As a practical measure the use of soft-glass rather than of Pyrex culture tubes is recommended for isolation purposes (shake cultures). They can be easily cut at the bottom, and the agar column then blown out into a sterile Petri dish. Numerous modifications are, of course, possible, but they do not affect the principle of the method in any way. By slicing the agar column the desired colonies can be laid free for examination and transfer. In case the colonies are of considerable size, it is often expedient to bend the agar column, as soon as a large colony extends beyond the cut end of the tube, against the wall. It will then usually split in such a way that the break passes exactly by the colony.

Even with the most homogeneous enrichment cultures as an inoculum, single colonies in the first series of shake cultures do not, as a rule, represent pure cultures. This is partly due to the fact that the purple bacteria, whenever they

develop in the proximity of the culture tube wall, tend to form a film along the side of the tube which is nearest the light. Hence, the entire outer surface of the agar column is frequently covered with a thin layer of organisms which may have developed from different cells of the inoculum. It is, however, a simple matter to prepare additional series of shake cultures from well isolated colonies. The mere dilution factor generally guarantees that tubes in a second series with not more than 10 to 20 colonies actually represent pure cultures. It goes without saying that a rigorous examination of all colonies in one tube must demonstrate the identity, at least in a morphological respect, of their contents before the conclusion may be reached that a pure culture has actually been achieved.

Another method for isolating pure cultures, sometimes useful in bringing out types which are present in the enrichment cultures in small numbers, consists in streaking on aerobic yeast agar plates. Naturally, only those purple bacteria capable of development under aerobic conditions will here grow into colonies. In such cases where the large majority of the *Athiorhodaceae* in a crude culture is represented by bacteria inhibited by oxygen, isolation of the potentially aerobic minority by means of shake cultures is well-nigh impossible, whereas the latter will be the only types developing aerobically. Often one can thus readily isolate "brown bacteria" from cultures which microscopically and by shake cultures appear to contain only red forms. If aerobic culture methods are used, it is immaterial whether incubation is in the light or in the dark.

The pure cultures are kept conveniently as stab cultures in yeast extract agar, and, when necessary, covered with paraffin seals. Many strains can be grown from the moment of isolation without this precaution; others can gradually become adapted to it. Incubation, at least for a few days, should be in the light, though after the cultures have developed sufficiently they may be kept in the shade or complete darkness. When kept in the light such cultures often remain viable for a long time; I have observed actively motile cells in cultures over 2 years old. But not all strains behave in this way; and if the cultures are to be used at definite times, it is best to make transfers about once every two or three months.

Aerobic representatives may, of course, be kept on ordinary slants and grown in the dark. However, it is my impression that under such conditions the photosynthetic ability of the organisms slowly weakens. I have had cultures of a number of strains both in stabs, exposed to continuous illumination, and on slants which were regularly kept in the dark except at times when they were transferred. In the course of 10 to 15 years the "dark cultures," though still capable of slow and scanty development under anaerobic conditions in the light, were decidedly less suitable for photosynthesis experiments than the corresponding stab cultures. By a process of selection one can succeed in gradually restoring the original vigor, but this takes time, and many transfers in media where growth depends upon photosynthetic activity are required to achieve it. So far, I have not observed a permanent loss of photosynthetic activity in cultures which have been grown in the dark for many years. Whether by careful selection subcultures might be isolated which have become non-photosynthetic remains a problem for the future.

4. GENERAL MORPHOLOGY OF THE NON-SULFUR PURPLE BACTERIA

"Würde man die Purpurbakterien auf Grund ihrer morphologischen Merkmale, die ja bei der systematischen Sonderung die erste Rolle spielen müssen, allein gruppieren, so würden sie sich über das ganze Bakteriensystem verteilen."

Molisch (4), p. 26.

I. Common morphological characteristics

The numerous strains of pure cultures which have been examined have certain morphological characteristics in common, apart from those which have already been enumerated in delimiting the group as a whole. Thus, all consist of motile bacteria in which the motility is caused by polar flagella; all are gram negative and none of them is capable of forming endospores.

a. *Mobility.* The designation of all strains as "motile" does not mean that all or most of the cells in a culture, even in a young one, exhibit motility. Although this is true for a majority of the strains when growing under favorable conditions, there are also those which upon a cursory examination would appear as immotile. These strains regularly give rise to cultures which are conspicuously mucilaginous; the developing cells produce a slimy sediment on the bottom of the culture vessel. Frequently the entire liquid is thus transformed into a highly viscous mass which, on being poured out of the container, appears to hang together in thick strands, much like ropy milk. The flow can be reversed by tilting the container at the proper angle, and what has previously been poured out will flow back into the flask or bottle. The vast majority of the individual cells in such cultures appears non-motile, and it is only on careful and prolonged examination that one can find an occasional bacterium which moves actively about.

No doubt the extensive slime production in such cultures must be held responsible for the scant signs of motility; the bacteria are glued together, as it were, in the massive strands which can be made to whirl up from the bottom into the supernatant liquid without losing their coherence. In these strands the individual bacteria are arranged more or less regularly, and this accounts in part for the high viscosity of the cultures. If the contents are vigorously shaken with a few glass beads, the strands can be broken up, and the organisms somewhat evenly dispersed. After this treatment the "ropy" property of the culture is lost completely. This behavior is strikingly reminiscent of the structural viscosity of ropy milk caused by *"Bacillus lactis viscosis"* Adametz (*Aerobacter aerogenes*), for which Kluyver (37) has shown the relationship between ropiness and structure of mucous strands.

Microscopic examination, especially of mounts in India ink, shows the arrangement of the purple bacteria in such strands, an arrangement which exactly fits Molisch's description of his *Rhodocystis gelatinosa* (4, p. 22): "Die einzelnen Zellen sind in dem Schleimhof nicht ganz wirr durcheinander, zwar auch nicht parallel, aber doch häufig vorherrschend nach einer Richtung gelagert ...."

Other strains of purple bacteria, both morphologically and physiologically different from the previous type, may form slime capsules without, however, giving rise to the formation of typical strands. Evidently the individual cap-

sules do not merge to an appreciable extent, and the bacterial cells, single or in chains, remain separate, without orderly arrangement. As a result of the mucus production, however, cultures of these organisms, too, usually contain a very small percentage of motile cells.

Although Molisch has described a number of species of *Athiorhodaceae* as "immotile," it is noteworthy that all these are characterized by slime formation. Czurda and Maresch (20) have observed motility in one of their cultures of a capsulated member of this group, but state specifically that it is of a transient nature.

Flagella stains of such cultures in which the majority of the bacteria appears motile are simple and give convincing evidence of the polar mode of insertion. It is far more difficult to obtain satisfactory preparations from the mucus-producing types. The best results have so far been obtained by placing a clump of the organisms at one edge of a large drop of water which is kept in the dark, while the opposite edge is exposed to light. The few motile cells accumulate, by phototaxis, in this region of the drop, so that after a while one can here find a much more favorable ratio of motile to immotile cells, with which a flagella stain can be attempted. In successfully stained slides these types also show clearly the presence of a single polar flagellum.

b. Gram stain. A great many slides of the various strains have, in the course of time, been stained by the Gram method. Cultures from stabs, slants, and liquid media of different composition have been used. In not a single case has any evidence been found for the presence of gram positive cells.

Molisch (4) does not mention the behavior of purple bacteria towards the Gram stain. On the other hand, Czurda and Maresch (20) report all their strains as gram negative, stating: "Gram-positive Athiorhodobakterien oder Gram-positive Inhaltsstoffe wie sie Schneider vorlagen, haben wir noch nicht angetroffen" (p. 120).

Apparently Schneider (22) is the only one who has claimed the existence of gram positive purple bacteria. In his description of the morphological characteristics of *Rhodobacillus palustris* one finds the following passage: "Dagegen sind junge in Teilung begriffene Bakterien .... ausgesprochen grampositiv; ihr ganzer Körper färbt sich nach Gram blauschwarz" (p. 93).

The discrepancy is due to the fact that Schneider did not, as he believed, use a pure culture of *R. palustris*. Also in a number of physiological respects the behavior of Schneider's cultures was so considerably at variance with that which I had observed with a number of strains of purple bacteria, tentatively identified as *R. palustris*, that it seemed desirable to compare the various isolates. Upon request Dr. Schneider obligingly sent me subcultures of his strain; and various experiments showed me that with these cultures his observations could be reproduced. It appeared, however, that the original cultures contained three kinds of *Athiorhodaceae*, in addition to representatives of the lactic acid bacteria, aerobic and anaerobic sporeformers, and non-photosynthetic *Pseudomonas* species. The gram positive organisms observed by Schneider, and reproduced in his fig. 2, belong to the lactic acid and aerobic sporeforming bacteria; the

purple bacteria, upon isolation in pure culture, behaved as gram negative organisms.

c. Absence of endospores. In the section dealing with the enrichment cultures it has already been remarked that inoculation of appropriate media with pasteurized materials has never yielded cultures of purple bacteria. Nevertheless, a few of the strains give, in certain media, an appearance which suggests the presence of endospores. But if such cultures are subjected to any one of the accepted methods for the specific staining of endospores (38, 46), the results are invariably negative.

It is doubtful whether the spore-like bodies, described by Schneider (22) as occurring in his cultures of *R. palustris*, are at all connected with the life history of purple bacteria. As stated before, Schneider's cultures were found to contain bacteria belonging to the groups of both aerobic and anaerobic sporeformers, so that the actual occurrence of endospores in some of his cultures would be expected.

Continued observations on strains which exhibit the above-mentioned morphological peculiarity have made it clear that the highly refractile inclusions distinctly resemble spores only under special conditions, and in fact bear no relation to reproductive structures but consist of oil or fat. This was first suggested by the appearance of the bacteria in media in which the continued growth gives rise to somewhat abnormal cell shapes ("involution forms"). In such cultures the refractile bodies showed up more numerously, while at the same time their irregular shape and size argued strongly against an endospore nature. The recent studies, especially those of I. M. Lewis (39–42), have proved the frequent occurrence of oil and fat inclusions in microörganisms, and with the aid of Sudan Black B (43) it was not difficult to ascertain that also in the purple bacteria the refractile bodies, whenever they occur, consist principally of fat.

Also, reproductive structures other than spores have never been observed in the purple bacteria.

These morphological features of the *Athiorhodaceae*, *viz.*, the occurrence of polarly inserted flagella, the negative outcome of the gram stain, and the absence of spore-formation in all strains, show that this group of organisms forms a remarkably homogeneous entity in a morphological respect. Were it not for the presence of the characteristic pigment system these properties would justify the inclusion of all *Athiorhodaceae* in the family *Pseudomonadaceae* as defined by Kluyver and van Niel (44) and adopted in the 5th edition of Bergey's Manual of Determinative Bacteriology. Thus it appears that it is no longer necessary to subscribe to the views of Molisch, quoted at the head of this section. The Viennese botanist believed that only by placing due emphasis on the occurrence of the pigment system could a haphazard distribution of the purple bacteria throughout the bacterial system be avoided. Considerations of this nature show that some definite achievements in the difficult problem of bacterial taxonomy have been attained.

How the various aspects of the morphology and physiology of the *Athiorho-*

dacrae can best be evaluated at the present time for the purpose of a more or less satisfactory classification of this group will be discussed in a later chapter.

II. *Differences in the morphology of different strains*

While in the previous pages the common morphological characteristics of the non-sulfur purple bacteria have been stressed, it is by no means true that all the strains of this group appear very much alike. In studying them by a variety of methods, it soon became possible to recognize a number of morphologically quite distinctive groups. These are chiefly distinguishable on the basis of the shape and size of individual cells, and by the color and general appearance of the cultures.

Fig. 1. *Rhodopseudomonas palustris*. Strain No. 52. Young (48 hr.). Culture in basal medium with 0.1% leucine; anaerobic; × 800.

Fig. 2. *Rhodopseudomonas palustris*. Strain No. 50. Culture in basal medium with 0.2% Na-crotonate; 7 days, anaerobic; × 800.

Fig. 3. *Rhodopseudomonas palustris*. Strain No. 18. Culture in basal medium with 0.2% glyceral; 10 days, anaerobic; × 800.

Considering the form of the cells alone, four types stand out clearly. They may be characterized as follows.

Type I. In young cultures the cells occur as small, short, slightly curved, and highly motile rods which show no tendency of becoming or remaining united in groups. As the cultures grow older, the rod-shaped cells become longer, frequently irregular, with bent or even branched forms conspicuously present. In this stage of the cultures the individual cells produce characteristic groups of somewhat star-shaped appearance. Such cultures present a close, though superficial, resemblance to *Corynebacterium* and *Mycobacterium* cultures (figs. 1, 2, 3).

Type II. Here also the individual bacteria in young cultures are small, short, and highly motile rods, but with an appearance of stretched cocci rather than vibrios. They exhibit a pronounced tendency to the formation of characteristic *chains*, resembling streptococci, or of irregular, long rods, the latter also frequently in strings of a zigzag arrangement (figs. 4, 5, 6).

Type III. While the microscopic aspect of young cultures is similar to that of Type II, the cells are still more spherical, and do not tend to the formation of chains. Rod-shaped structures are seldom encountered, and, when occasionally found, appear as typical "involution forms" (Figs. 7, 8).

Type IV. This is the most conspicuous morphological group, and comprises the strains in which the cells are spiral in shape (Figs. 9, 10).

Correlated with these differences in cell form are dissimilarities in the general appearance of the cultures. For example, the cultures of types I, III, and IV

are not, those of type II are usually mucilaginous. Also color differences exist which are consistent and characteristic for these groups.
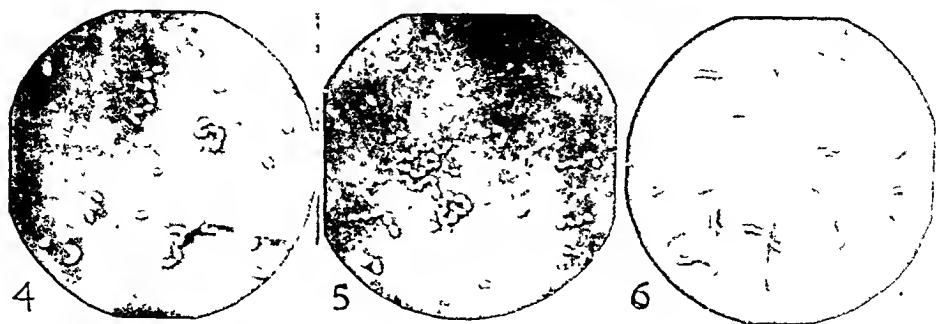
FIG. 4. *Rhodopseudomonas capsulatus*. Strain No. 42. Culture in basal medium with 0.2% Na-*iso*-butyrate; 3 days, anaerobic; × 800

FIG. 5. *Rhodopseudomonas capsulatus*. Strain No 32 Culture in basal medium with 0.2% Na-glutamate; 5 days, anaerobic; × 800.

FIG. 6. *Rhodopseudomonas capsulatus*. Strain No 32 Culture in yeast extract. 0.5% phosphate, pH 7.0; 6 days, anaerobic; × 800.
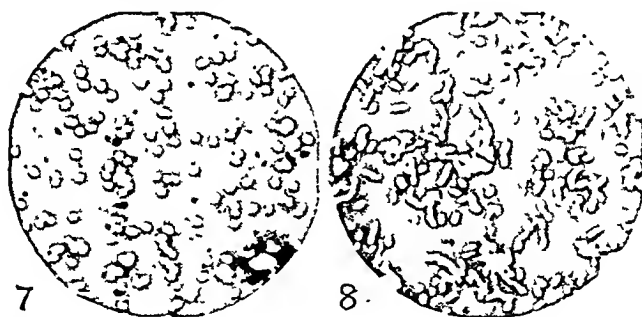
FIG. 7. *Rhodopseudomonas sphcroides*. Strain No. 36. Culture in basal medium with 0.2% ethanol; 5 days, anaerobic; × 800.

FIG. 8. *Rhodopseudomonas sphcroides*. Strain No. 36. Culture in basal medium with 0.1% Na-*n*-valerate; 7 days, anaerobic; × 800.
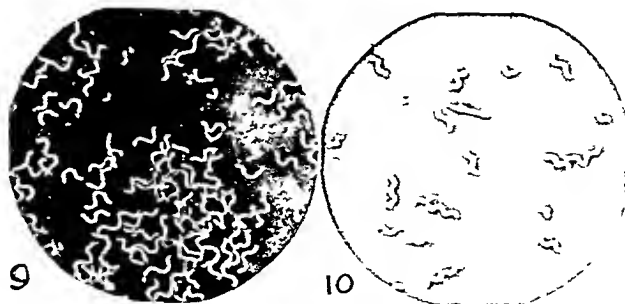
FIG 9. *Rhodospirillum rubrum*. Strain No 11. Culture in yeast extract. pH 7, 2 days, anaerobic; × 800.

FIG. 10. *Rhodospirillum rubrum*. Strain No 4 Culture in yeast extract pH 6 0; 4 days, anaerobic; × 800

Two aspects of the coloration of the cultures must be distinguished: the pigmentation of the cell masses themselves, and the development of color in the

surrounding medium. It is often tacitly assumed that the purple bacteria are exclusively "chromophorous" in the sense of Beijerinck (47), i.e., that the pigment is intimately associated with the cell constituents, does not diffuse out of the cells, and fulfills a physiologically important function. Though in my experience this is true for the *Thiorhodaceae* which have so far been studied in pure culture, it does not hold rigorously for all the members of the group of non-sulfur purple bacteria. Both in liquid cultures and in agar stabs, one can readily observe that the development of certain strains is accompanied by a bluish-red discoloration of the medium. In sufficiently old liquid cultures, where the bacteria have settled on the bottom, it is not uncommon to find the initially colorless supernatant liquid colored a bright and perfectly transparent purplish-red. Stab cultures of the same strains show progressive diffusion of a similar coloring matter from the region of growth until, after some weeks of development, the entire medium is colored.

In the beginning I ascribed this phenomenon to a slow autolysis of the bacteria with the liberation into the environment of the protein-pigment complex first isolated and described by French (33, 45) as "photosynthin." The strains could, on this supposition, be segregated into a. those readily subject to autolysis, and b. stable ones. The possibility of the occurrence of autolysis is, especially in media which during development become strongly alkaline, far from remote, and autolysis had been demonstrated by Muller (7) in cultures of *Thiorhodaceae*. However, during the course of the investigations it became evident that the colored culture solutions cannot be explained on this basis; they are due to the production of a water-soluble pigment, very different from photosynthin, and about which more will be said later. Characteristic for these solutions is the absorption spectrum; it lacks the typical bacteriochlorophyll band at 590 mμ, but presents instead two new bands, not encountered with any of the known *Thiorhodaceae*, at 610 and 565 mμ. The strongest absorption by the solutions occurs around 535 mμ.

The excretion of this pigment occurs frequently in cultures of the morphological types I and II, whereas it has never been encountered in types III and IV. Its extent varies considerably, but seems in general related to the density of the cultures. Media which support extensive growth usually become deeper colored than those which give rise to only a faint development of the bacteria. It can, however, be detected wherever the organisms grow.

Striking also are the color differences of the cultures due to the pigmentation of the organisms themselves. But here a considerable range of shades may be observed, even with one and the same strain, depending upon the composition of the medium and the culture conditions. The extremes which I have observed with the different strains, and in a large variety of media, are a pale brownish-yellow, practically straw-colored mass of bacteria, and an intense, deep bur-gaudy red. In between, a number of color types becomes distinguishable as more or less characteristic for certain strains. Special mention should be made of a group that normally develops with a sediment the shade of which can best be described as "peach-colored." The color is pale and very delicate; it is

reminiscent of the appearance of certain *Thiorhodaceae* in media containing sufficient sulfide, so that the individual cells are stuffed with sulfur globules, whereby the pigment system of the organisms is partially masked. Closely correlated with this type of pigmentation in the *Athiorhodaceae* is the mucilaginous nature of the growth, discussed before in connection with the motility.

On the basis of anaerobic cultures alone, two more groups of strains can be segregated. In cultures of the first group the growth appears red, varying with the medium from a deep brownish-red to a lighter shade with a faint bluish hue. The preponderance of red in these strains sets them clearly apart from that group which generally appears brown, with a dominant yellow. But this distinction is not an absolute one, as is shown by an examination of aerobic cultures of strains belonging to the latter type. If development occurs in the presence of air—and it should here be emphasized that this is not always the case; some of the strains appear to be strict anaerobes—it often presents a color which is very similar indeed to that of cultures of the first group.

While studying various representatives of the *Athiorhodaceae* Molisch (4) had noticed that not all cultures exhibit the same color. His spectroscopic investigations of extracts of the pigments led him to the conclusion that the

TABLE 2

Absorption maxima of various representatives of Athiorhodaceae in the visible region of the spectrum

Absorption maxima, millimicrons

Group 1............................	590	550		515	
Group 2............................	590		530		500
Group 3............................	590			520–510	

differences must be ascribed to the existence of two kinds of red pigment, which he designated as "bacteriopurpurin α" and "bacteriopurpurin β." The former was obtained from a pure culture of *Rhodobacillus palustris*, the latter from a *Rhodospirillum* species. The nature of these pigments will be discussed in more detail in a separate section later on; it may here be briefly stated that Molisch's contention of the existence of more than one "bacteriopurpurin" has been amply verified by more recent studies. It is now quite certain that more than two pigments of this type occur in the non-sulphur purple bacteria.

In general it can be stated that a spectroscopic examination of the intact, living cells of representatives of the group reveals three types. All three show an absorption band centering around 590 mμ which, as will be shown later, is due to the green component of the pigment system, the bacteriochlorophyll. The differences in the absorption spectra occur in the shorter wavelength region. The main characteristics of the three types appear from Table 2. The figures should be considered as approximations; a considerably greater degree of variation seems to exist. However, it is such an easy matter to observe these bands with a simple hand spectroscope that, for a general orientation, they have proved useful.

Those strains which are indubitably red all appear to contain the pigment responsible for the absorption at 550 mμ. It is of importance to note that the typical "brown bacteria" which give rise to red cultures when grown in the presence of air also show this most characteristic absorption band in the red phase, whereas anaerobic cultures of the same organisms belong spectroscopically to group 3. I wish to emphasize that the spectroscopic groups should not be confused with the four morphological types distinguished previously. The spirillum strains, for example, which have been designated as belonging morphologically to Type IV, can be separated into two groups on the basis of their spectroscopic characteristics (groups 1 and 2), while also in each of the other morphological types spectroscopic differences can be observed.

Although cultures of one strain in a variety of media may present different shades when viewed with the naked eye, the determination of the main absorption maxima shows that the color variation in such series should perhaps be ascribed to differences in the relative amounts of the various pigments, rather than to the occurrence of different pigments. The only exception to this generalization, already noted, is the production, under aerobic conditions, of a red pigment by some strains which do not appear to manufacture it in the absence of air.

III. Morphological variation in the non-sulfur purple bacteria

From the foregoing remarks concerning color variation in pure cultures exposed to different conditions, it follows that one cannot adequately characterize the color of a single strain by assigning to it a certain number from one of the existing color codes. Such a procedure would be entirely misleading unless at the same time the culture conditions were rigorously specified. The same holds true, and in some cases in a spectacularly exaggerated manner, for the morphological characteristics of any one strain. The reaction of the medium, the nature and concentration of the nutrient materials present, the culture conditions in general, exert a distinct influence upon the morphology of the organism. For this reason it is mostly impossible to interpret previous descriptions of representatives of the *Athiorhodaceae*, based largely on observations with impure cultures, in terms of identifiable species. Also here a rigid standardization or specification of environmental conditions would be required in order to furnish a brief characterization which could lay claim to accuracy and usefulness. An alternative solution consists of carrying out extensive observations on the relationship between morphology and environment, and incorporating the results in the description of the various morphological types.

Similar difficulties were encountered during the study of the *Thiorhodaceae* (3). They ultimately led me to postpone an attempt at a more satisfactory description and classification of this group until more extensive information was available. Such an attitude seemed sufficiently justified because the strains available for pure culture studies appeared to comprise only a very small number of morphologically distinctive groups, whereas collections from naturally occurring mass cultures often present a far greater abundance of morphologically recognizable

types. Of decisive importance in this respect was the fact that the most conspicuous of the species, described from observations on impure materials, had not yet been obtained in pure culture.

The situation in the case of the non-sulfur purple bacteria is, fortunately, quite different. Not only is the number of strains, available as pure cultures, vastly greater, but in addition it is my well-considered opinion that among these are represented all the types that have previously been more or less clearly recognized and described.

To this should be added yet another circumstance which argues strongly in favor of the contention that the general biology of the *Athiorhodaceae* is at present better understood than that of the purple sulfur bacteria. It has already been mentioned that by a judicious selection of enrichment methods it is now possible to obtain almost any one of the known representatives of the first group from a crude inoculum. Our current interpretation of the results of enrichment cultures leads to the conviction that the medium in which a certain microorganism or group of microörganisms gains predominance over the other types present in the inoculum, represents fairly accurately the conditions under which also in nature this particular group will be found in active development, in other words, corresponds to its "natural environment." The more restricted the specific microflora of an enrichment culture, and the simpler the composition of the medium, the closer will be the approximation. Hence the successful enrichment of the different members of the non-sulfur purple bacteria in media which do not support growth of other microörganisms makes one feel confident that the natural environment of these organisms has been closely approached. And this, in turn, implies that one can better evaluate the normal range of variation that each specific representative is likely to display in its natural habitat. On the other hand, since many types are found simultaneously in some of the more complex media, and their ultimate identification will have to be based on pure culture studies, it is equally necessary to pay close attention to their behavior in media which do not necessarily correspond in composition to the simplest and most specific ones.

Some of the more striking examples of morphological variability will be briefly indicated at this point; a more detailed discussion will be found with the description of the various species.

In the first place the spirilla. They are so easily recognizable, and so conspicuous that the generic diagnosis is, indeed, a simple matter. But when it comes to distinguishing or identifying species, the matter is considerably more complicated. In the relatively few studies on members of this genus (41, 48, 49), it has been customary to differentiate and describe species on the basis of the dimensions of the cells, and of the turns. The extent to which such a procedure will yield satisfactory results obviously depends upon the constancy of these characteristics. A glance at figures 11–16, illustrating the morphology of one single strain in a number of different media, will show at once how extraordinary and unexpected are the variations in this respect. Not only the length and width of the turns, and the number of turns of an individual (not necessarily a

single cell!), but also the size and shape of the bacterium itself may be so different for cultures of the same strain in various media that one would be tempted to assign the organism to a number of different species, depending upon the culture examined. And yet, it is not possible to designate any one of these morphological types as "normal," and the others as "abnormal." This is, perhaps, most adequately illustrated by the results experienced during the isolation of various strains from enrichment cultures.
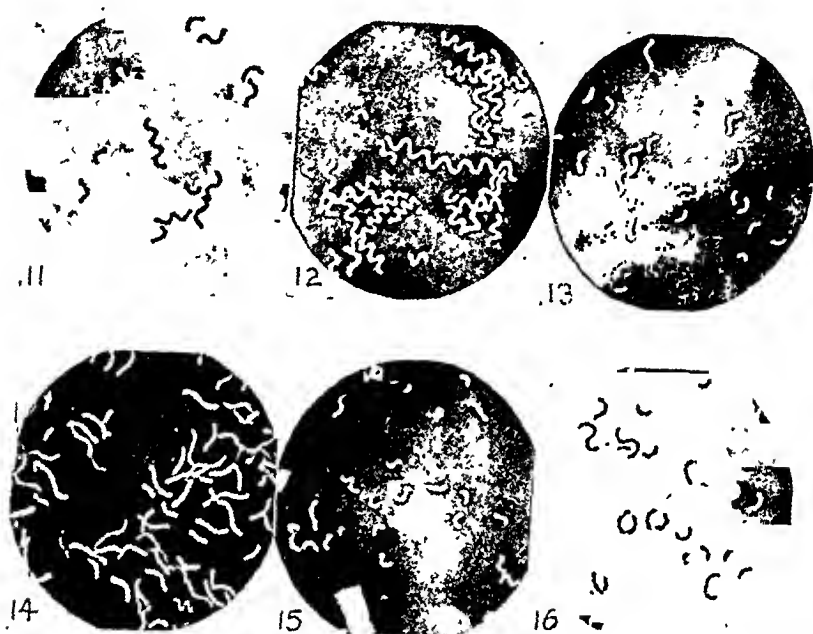
FIGS. 11-16. *Rhodospirillum rubrum*, Strain No. 8; × 800; 7-day anaerobic cultures in basal medium with:

11	0 2% Na-acetate
12	0 2% Na-propionate
13	0.2% Na-*n*-butyrate
11	0.2% Na-fumarate
15	0 2% Na-maleate
16	0 2% Na-aspartate

In a variety of simple media, differing chiefly in pH or in the organic substance used, spirillum species were regularly encountered, and often constituted the majority of organisms present. The most interesting phenomenon was that the different media seemed to contain such strikingly divergent types. When pure culture isolations were attempted with the use of shake cultures in yeast *agar* the original expectations were, however, sadly shattered. To be sure, from all the enrichment cultures in which characteristic spirilla abounded, pure cultures of some spirillum were easily obtained. But the spectacular differences no longer appeared. The crude culture might have contained extremely large organisms, or beautifully and tightly wound corkscrews, or cells resembling

rings; the pure colonies, upon inspection, showed no observable difference from what has been known for half a century as *"Spirillum rubrum."* Repeated transfers of the enrichment cultures to liquid media of the same composition, on the other hand, revealed that the spectacular forms persisted. And the later experiments with the isolated pure cultures of *"Spirillum rubrum"* provided the final and unambiguous answer to the problem why the unusual spirillum types were not found in the yeast-agar shake cultures; they are typical only for certain media and will reappear upon inoculation into the media in which they were first observed.

Equally characteristic, although not so spectacular changes in morphology have been observed with many of the other stains, in which the general morphology is not that of a spirillum. For example, the rather pronounced vibrio-shape of some strains can be observed clearly in some specific media only; in others these organisms grow as more or less long rods. (See figs. 1–3). Other strains, again, may occur as very short rods, resembling streptococci when they remain attached, or under different conditions, as long, irregular rods. (See figs. 4–6).

It will thus be clear that an adequate morphological description of the *Athio-rhodaceae* must comprise a characterization of the organisms as they develop under a variety of environmental conditions. Naturally, the selection of such conditions as might be regarded more or less normal and significant is, in part at least, a matter of personal appreciation. In the following chapter a condensed review will be given of the general physiology of the group. From this it will become apparent what, in my opinion, should be considered as the fundamental aspects of the vital activities of the organisms. This, in turn, permits a decision as to the nature of the general habitat of the group and its representatives. Upon the criteria so developed can then be based an evaluation of the significance of the pattern presented by the morphology of the individual strains as influenced by environmental conditions.

5. GENERAL PHYSIOLOGY OF THE NON-SULFUR PURPLE BACTERIA

"Da die Ernährungsphysiologie der isolierten Stämme noch nicht entsprechend geklärt ist, bleibt vorderhand unbekannt, welche der isolierten Stämme zusammengehören."

Czurda and Maresch (20), p. 106.

I. The influence of light and of oxygen on the development of the group

In spite of the nutritional studies, carried out on this group of organisms during the past 35 years, a perusal of the literature pertaining to the subject leaves one with a feeling that the existing information is extremely fragmentary and confusing. A partial explanation for this situation is not difficult to find; the *Athiorhodaceae* comprise a relatively large group of bacteria, and the individual characteristics of various types show, in a number of respects, quite considerable differences. Even so, it should be possible to develop a more integrated and satisfactory picture of the general aspects on the basis of a clearer concept of the biochemical activities of the organisms.

A good deal of the confused state of the problem can be ascribed to the fact that for a long time the rôle of light and of oxygen in the physiology of the purple bacteria was not clearly understood. That a supply of radiant energy is not always essential in order to secure development was known to Molisch who wrote:

"Zwar ist bei Reinkulturen, soweit meine Untersuchungen reichen, das Licht nicht notwendig, denn es können manche Rhodobakterien namentlich in festen Nährböden auch im Finstern sehr gut wachsen, allein in flüssigen Nährmedien ist der Einfluss schon leicht erkennbar. Besonders auffallend macht sich die Einwirkung des Lichtes im Wasser mit faulenden organischen Stoffen geltend, da ein reichliches Aufkommen oder das Auftreten der Purpurbakterien überhaupt an die Anwesenheit von Licht gebunden ist" (4, p. 72–73).

For many years thereafter the question whether light was "necessary" or merely "stimulating" was an ardently debated point. As late as 1933 Hama (50), in commenting on the matter, wrote:

"Bei meinen Versuchen wurde in der im dunklen Brutschrank bei 25°C. gehaltenen Kultur keine Entwicklung von Spirillen beobachtet. Aber natürlich sind für eine endgültige Lösung dieser Frage vollständigere Untersuchungen mit der Reinkultur erförderlich" (p. 143).

Also the problem of the effect of oxygen on the development of these purple bacteria has occupied a number of investigators, resulting in contradictory statements. Here again, the first definite pronouncements came from Molisch. Studying a variety of forms of *Athiorhodaceae*, he arrived at the conclusion that not all species react in the same manner. Some types behave as ordinary aerobic bacteria, whereas others appear to be strict anaerobes. A disregard of this divergent behavior of different strains on the part of later workers, and especially the frequent use of impure cultures, have added to the confusion.

As a more integrated concept of the fundamental physiology of the purple bacteria was developing, it became increasingly probable that there would exist a close connection between the influence of light and of oxygen on their development. The available evidence pointed strongly to a photosynthetic activity in illuminated cultures, but the failure of such cultures to produce oxygen in detectable quantities, even if Beijerinck's exceedingly sensitive "luminous bacteria method" (51) was used, indicated that the photosynthetic process was in some way different from that of the green plants. For a more detailed discussion of this problem the reader is referred to the recent reviews of the bacterial photosyntheses (8, 52, 53); suffice it to state here that these processes came to be considered as variants of a general photosynthetic reaction which can be expressed by the equation

$$CO_2 + 2H_2A \xrightarrow{\text{Light}} (CH_2O) + H_2O + 2A.$$
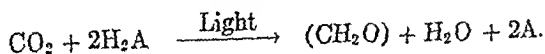
This equation implies an ability on the part of the organisms of synthesizing cell material (in the equation indicated as (CH_2O), a first, crude approximation) from carbon dioxide in the light, in the presence of an appropriate hydrogen donor, H_2A, the nature of which may differ for various organisms. Since oxygen will be evolved only in case the H_2A function is fulfilled by H_2O, it follows that in the presence of oxidizable substances, such as are required by the purple

bacteria for photosynthetic activity, an oxygen production will not take place in cultures of these organisms.

The non-sulfur purple bacteria seemed, according to previous investigations, to require organic substrates for growth. Supposing that these materials would play the role of H_2A in a photosynthetic reaction, it could be inferred that growth of the purple bacteria would be possible under strictly anaerobic conditions, since oxygen does not enter as a factor in the photosynthetic process. But the supply of radiant energy would be a prerequisite.

Numerous experiments have amply confirmed this. All purple bacteria are, indeed, capable of development in the complete absence of oxygen, provided the cultures are properly illuminated. On the other hand, none of the many strains of *Athiorhodaceae* has ever developed anaerobically in the "dark."

It is necessary to specify what is meant here by "dark." The photosynthetic pigment apparatus of the purple bacteria contains a bacteriochlorophyll which, though chemically closely related to green plant chlorophyll, has a distinctly different absorption spectrum. Most characteristic are the absorption maxima in the near infra-red region (800–900 mμ), and the absorbed infra-red radiation is photosynthetically functional (5, 31, 32, 33, 54). It is, consequently, possible to obtain growth of purple bacteria under anaerobic conditions if the cultures are exposed to a source of infra-red radiation in this region. From the point of view of the purple bacteria this is, however, equivalent to incubation in the light. When, therefore, in the following pages the designation "dark" is used it implies the absence of effective radiation, including the near infra-red region, rather than the visible region only.

With the demonstration that the supply of radiant energy is essential for development of the purple bacteria under anaerobic conditions, the situation has, nevertheless, been clarified only in part. For the question still remains to be answered whether any of these organisms can, as has been claimed by various investigators, develop in the dark. In view of what has been said above with respect to the effectiveness of infra-red radiation for photosynthesis, it might be assumed that positive results of non-illuminated cultures should be interpreted as resulting from the failure to eliminate this possible source of radiant energy.

Although it will not be denied that in some cases this factor may have been responsible for the observation of development, it is by no means true that growth in the dark is always caused by short-wave length, infra-red rays. The explanation is again furnished by an examination of the general equation for photosynthesis. This can be paraphrased by stating that it represents a reaction in which an oxidizable substance, H_2A, is dehydrogenated with carbon dioxide acting as the ultimate hydrogen acceptor. In this form the reaction differs from an ordinary oxidation only in the type of acceptor used. It might, therefore, be expected that those purple bacteria which are not adversely affected by oxygen should be able to carry out the dehydrogenation of H_2A also with oxygen as the final acceptor. And, because such oxidations are exergonic,[1] a supply of radiant energy would not be required for the process, in contrast to what holds for the reduction of carbon dioxide with the same substrate. Furthermore, since H_2A is present in the form of an organic substance, and so many oxidative

[1] "Exergonic" is used in preference to exothermic; see Coryell (55).

(aerobic) microörganisms are capable of development as a result of such oxidation reactions, it would be entirely in keeping with our current knowledge if the non-sulfur purple bacteria were to show the same behavior. Theoretically, then, the development of those representatives of this group which can live in the presence of air should also be possible in the dark, but under aerobic conditions.

This, again, has been amply confirmed by numerous culture experiments. The most convincing are those which demonstrate that one and the same strain, inoculated into one and the same medium, grows in darkness only if the conditions are aerobic, but not in the absence of oxygen. Such results effectively rule out the possibility that infra-red irradiation would have made development possible, for if this were the case, then growth would also have taken place in the anaerobic cultures.

The situation can therefore be summarized as follows: All members of the non-sulfur purple bacteria can grow under strictly anaerobic conditions, but only when properly illuminated. Some representatives can also develop in darkness; this is, however, possible only under aerobic conditions, and hence becomes a property restricted to representatives which are not strict anaerobes.

In the course of the studies on the *Athiorhodaceae* several observations have been made which demonstrate that the behavior of individual strains toward oxygen does not always constitute a fixed property. Especially cultures of purple spirilla show a remarkable degree of variability in this respect. It has frequently been found that a recently isolated culture of an organism, bearing a close resemblance to the well known *Spirillum rubrum* Esmarch, behaved like an obligatory anaerobe. There are, however, a number of more or less authentic strains of Esmarch's organisms which have been successfully maintained as pure cultures on slants, i.e., under aerobic conditions. The latter can consequently be grown in the dark, while the new isolates, in agreement with the previous remarks, must be cultured anaerobically, and thus develop only in the light. Stab cultures of these strains in yeast agar give rise to growth only in the lower part of the agar column, the upper part (± 2 cm) remaining devoid of growth unless the medium is covered with a seal immediately after inoculation. It may even occur that a stab culture does not develop at all unless it is sealed. After a number of transfers, particularly if made in fairly rapid succession, and using a heavy inoculum, such strains appear to become less and less adversely affected by oxygen, and ultimately transfers to aerobic slants become possible. Admittedly, the organisms behave as "micro-aerophils," but this is true also of the typical *Spirillum rubrum*. Similar experiences with initially strictly anaerobic bacteria which could gradually become adapted to live in the presence of air have been reported by Prévot in his studies on anaerobic streptococci (56).

Transitions of this kind have been observed with many other strains of non-sulfur purple bacteria. Nevertheless, it must not be inferred that it will invariably be possible to bring about such adaptations by experimental means. There are still a few strains in my collection which must be treated as obligatory anaerobes. And these, in consequence, cannot yet be grown in the dark.

The observations of Molisch concerning the effect of light on cultures of

Athiorhodaceae, quoted earlier in this chapter, can now readily be explained. Those cultures in which plant and animal remains are undergoing decomposition under a deep layer of liquid must, of course, be considered as strictly anaerobic with the exception of a very shallow surface film. Since, in such experiments, the latter is always occupied by a dense growth of aerobic microörganisms, the purple bacteria are unable to make their appearance unless the cultures are exposed to light where anaerobic development of the brown and red organisms becomes possible.

More involved is an adequate interpretation of the results obtained by Schneider (22). As has been mentioned earlier, his experiments were conducted with impure cultures, containing a variety of non-photosynthetic bacteria in addition to members of the *Athiorhodaceae*. Hence the composition of the medium and the conditions of incubation must have played a decisive rôle in determining which of the various microbes present in the inoculum would develop. A more detailed analysis of his observations will be taken up in the next section. Also the effect of light and air on the pigment production by the purple bacteria will be treated later.

II. *The nutrient requirements of the non-sulfur purple and brown bacteria*

a. General considerations. It need not be argued that an exact study of the nutrient requirements of any microörganism can be carried out only with pure cultures. On the other hand, it is frequently possible to derive important inferences from observations with mixed cultures which may give clues concerning the problem of nutrition, and which it would be difficult to reach otherwise, except perhaps on the basis of a systematic investigation of such a scope that it requires more than ordinary facilities and courage to carry to completion.

Such a situation is illustrated with remarkable clarity by our present knowledge of the nutritional physiology of the non-sulfur purple bacteria. When Molisch achieved pure cultures of a number of representatives of this group (4), he carried out some experiments with a view to finding a medium in which they could be grown satisfactorily. Although the number of different media employed was relatively large, their composition cannot be said to have covered a particularly wide range. Nor is it evident that in planning these media Molisch was guided by considerations of the conditions prevailing in the crude cultures where purple bacteria abounded.

Summarizing the results of experiments with two different pure cultures in media prepared from river- or sea-water by the addition of sucrose, dextrin, inulin, asparagine, glycerol, ammonium tartrate, or peptone, or simple mixtures of these compounds, Molisch concluded:

"Keine Entwicklung oder nur eine minimale findet statt in den Gefässen, die im Moldau-wasser Rohrzucker, Dextrin, Asparagin, Glyzerin, weinsaures Ammoniak, Pepton oder Gemische von Asparagin mit Dextrin, Asparagin mit Glyzerin und Dextrin mit Inulin enthalten.

Hingegen zeigte sich eine reichliche Entwicklung in absteigender Reihe in den Gemischen von Pepton-Glyzerin, Pepton-Dextrin und Pepton-Inulin. Nirgends war die Vermehrung so üppig wie bei Pepton-Glyzerin, es erwies sich daher unter sämtlichen in der Tabelle

angeführten Nährlösungen diejenige, welche aus Moldauwasser mit 1 Proz. Pepton und ½ bis 1 Proz. Glyzerin bestand, unter meinen Versuchsbedingungen als die beste Nährlösung" (4, p. 66).

These pioneer investigations have been of decisive influence on the later studies of the nutritional physiology of the *Athiorhodaceae*. The "peptone-glycerin medium" of Molisch has been used by all subsequent workers, though occasionally it was modified to some extent. As late as 1937, Nakamura (57, 58) used the old formula; and Czurda and Maresch (20), the only investigators who have recently attempted to study the nutrient requirements of the non-sulfur purple bacteria in more detail, make the claim:

"Für eine erfolgreiche Fortzüchtung musste der Nährboden unbedingt 1% Pepton enthalten, eine Feststellung, die sich Mit der von Molisch, Schneider und Muller deckt. *Phaeomonas Nr. 23* bildet insofern eine Ausnahme, als es, wie noch ausgeführt werden wird, nur auf Mineralsalznährböden gedeiht. Ein Zusatz von 1% Glyzerin oder 1% Glucose hat sich bei den Kulturen unter vermindertem O_2-Druck zwar als zweckmässig erwiesen, jedoch keine auffallende Steigerung der Vermehrungsintensität oder des Ertrages herbeigeführt" (p. 107).

Obviously the complexity of such media, which may serve an excellent purpose in maintaining pure cultures, or in growing large quantities of the organisms, does not lend itself to a ready interpretation of the nutritional physiology of the organisms. Further remarks in the publication of Czurda and Maresch certainly do not ameliorate the situation. Some of their experiments, particularly with media containing peptone or yeast extract to which the sodium salts of various organic acids had been added, yielded results which apparently were not in agreement with those obtained by Gaffron (1, 5). Since the last-mentioned investigator had not used pure cultures, Czurda and Maresch, evidently unaware of the fundamental significance of Gaffron's experiments for which pure cultures were not required, concluded:

"Diese Festellungen legen neuerdings die Vermutung nahe, dass die Ergebnisse von Versuchen mit unreinen Zellgemischen durch die Mitwirkung der anderen Bakterien getrübt werden" (20, p. 108). Indeed, the impression gained from the existing literature leads to the conviction that the published reports on the nutrient requirements of the Athiorhodaceae are inadequate to lay a satisfactory foundation for a better understanding of the problem, a conviction which is emphatically supported by the following statement: "Es hat sich später gezeigt, dass das Problem der Kultur der *Athiorhodobakterien* nicht mit Hilfe einer bestimmten Nährstoffmischung gelöst werden kann" (20, p. 108).

So pessimistic an outlook is hardly justified by the facts. On the one hand it is a simple matter, as has been pointed out before, to grow the members of this group in complex media, of which I have found yeast autolysate the most satisfactory. On the other hand, a consideration of the conditions under which these organisms develop in crude cultures helps greatly in formulating a working hypothesis concerning their more exact nutrient requirements.

In the course of some early experiments with non-sulfur purple bacteria I had observed that in sugar-containing media these organisms appeared to develop much better if lactic acid bacteria were simultaneously present (3, p. 102).

Subsequently it was established that salts of various organic acids can be used readily by members of the *Thiorhodaceae*. Muller (7) then succeeded in demonstrating that this utilization is due to the occurrence of a photosynthetic process in which the hydrogen-donor function (H_2A in the equation previously given) is fulfilled by the organic substance. In the meantime Gaffron, using the manometric technique, had shown that *Athiorhodaceae* can carry out a photosynthetic reaction in the presence of various fatty acids. ((1); Gaffron did not publish his results until 1933, but he had already presented them at a meeting of the A. A. A. S. in Pasadena in 1931.) It was thus evident that, at least in the presence of organic substances, the fundamental metabolic activities of the two groups of purple bacteria are very similar.

Let us now turn our attention to the processes occurring in a satisfactory enrichment culture for these organisms, prepared according to the methods of Molisch, Buder, and others. At the outset, the organic matter is deposited on the bottom of the container, there to undergo a gradual decomposition. Except for the surface layer, the conditions in the culture vessel will very soon be anaerobic. We may, therefore, confine ourselves to a consideration of the anaerobic degradation of the substrate. The numerous experiments on such decomposition processes of complex organic materials leave no doubt as to the nature of the chief decomposition products. One may safely claim that they will consist of (a) gases, mainly carbon dioxide, hydrogen, and methane; (b) dissolved inorganic substances, among which hydrogen sulfide and ammonia are predominant; and (c) organic compounds, mostly belonging to the groups of simple alcohols, fatty acids, hydroxy- and dibasic acids, and amines. It is, of course, quite possible that also other groups of substances, including more complex ones, may temporarily accumulate to some extent. But their presence will be transitory.

Are the purple bacteria involved in these primary decomposition processes? At first the answer to this question would seem to be undeniably affirmative for the simple reason that they always develop in such cultures. But on second thought this becomes very doubtful. There are three important observations that clearly do not conform with this view. The first one is concerned with the time at which the purple bacteria appear in macroscopically visible numbers. In the experiments of Molisch, Buder, and others, in short, before continuous illumination was used for securing enrichment cultures of purple bacteria, these organisms usually were found, in sufficient numbers to color the liquid, only after one to two weeks. Now it is a well-known fact that the decomposition of the plant and animal remains sets in considerably earlier, and in the course of a few days has already reached an appreciable extent. Hence the fact that the purple bacteria are not immediately found indicates strongly that they are not concerned in the primary decomposition of the complex materials. This is supported by the distributions of the red organisms in the vessels. They do not occur in close contact with the organic matter originally introduced, but in the liquid above, sometimes coloring the entire contents of the container up to the surface. In the early stages one frequently finds them as delicate clouds,

several centimeters above the deposit on the bottom. Since most of the recommended materials for securing enrichment cultures of purple bacteria are insoluble in water, this observation leads directly to the conclusion that these organisms develop by utilizing substances in solution, rather than by decomposing the initial substrate. And, thirdly, microscopic examination of such cultures shows that representatives of the *Thiorhodaceae* generally develop side by side with members of the *Athiorhodaceae*. The appearance of the former, stuffed with sulfur globules, shows convincingly that they, at least, are growing at the expense of hydrogen sulfide. For this group, then, it must be admitted that its development constitutes a secondary flora and that its appearance is made possible by the occurrence of decomposition processes which give rise to the formation of hydrogen sulfide, a thesis which was eloquently defended by Winogradsky (10) as early as 1887!

The above considerations make it seem inevitable to deduce that also the non-sulfur purple bacteria occur in the enrichment cultures as a secondary flora, depending for their growth on the production of simple breakdown products formed by a primary, and varied, microflora from the raw material used. In this connection the experiments of Gaffron, demonstrating the rapid utilization of a large number of fatty acids by *Athiorhodaceae*, gain an added significance because this class of substances would undoubtedly constitute an important fraction of the primary decomposition products. In particular must this be true in decomposing oils and fats where Seliber (65) and Pigulewski and Charik (66) had found purple bacteria growing in profusion. The development of the non-sulfur purple bacteria in the enrichment cultures could thus be ascribed to the gradual accumulation of various primary decomposition products, simple in nature, which would be utilized extremely efficiently by the photosynthetic mechanism previously described. It should then be possible to culture these organisms in media of a very simple composition, analogous to those used for growing the purple sulfur bacteria, but containing organic substances instead of sulfide.

Various attempts have been made in the course of the past several years to verify this. Although it has thus far been impossible to secure satisfactory development with any one of the numerous strains except in the presence of complex substances, such as peptone or yeast extract, this does not imply that the large quantities of these substances, used since Molisch's studies, are required. Under proper conditions surprisingly small amounts suffice.

Prior to 1935 it was generally believed that a bacterium, unable to develop in a medium containing an ammonium salt as the only nitrogen source, but which would grow profusely in media with peptone or meat extract, thereby demonstrated that the organism could not synthesize the various amino acids which are part of its protein structures. Since the beautiful investigations of Knight *et al.*, the Lwoffs, W. H. Peterson and co-workers, and many others, it has become clear that this is not necessarily the case. The ability of even such fastidious organisms as the propionic and lactic acid bacteria to utilize ammonia nitrogen to a large extent for synthesizing many of their proteinaceous components from this simple nitrogen source has been firmly established. This, together with the demonstration that the special growth factors required by such organisms are often identifiable with a

particular amino acid or with the prosthetic groups of various enzymes has given rise to the idea that, in microbes as in the metazoa, special growth factors or vitamins fulfill the same function, *i.e.* that of supplying essential constituents which the organism in question cannot itself manufacture from ammonium salts in the presence of an otherwise suitable substrate. If this special constituent contains nitrogen, it may still be maintained that the organism requires "complex nitrogen compounds"; but the meaning of this statement has lately undergone a considerable change.

These recent developments in our understanding of the complex nutrient requirements of microörganisms make it readily conceivable that yeast extract and similar materials contribute one or more growth factors which the *Athiorhodaceae* are unable to synthesize themselves. It is easy to understand that such compounds would regularly be present in the natural environment of the organisms, which always live together with a host of non-photosynthetic bacteria of different types and nutrient requirements. Hence, even if the raw material undergoing decomposition through the activities of the primary flora does not contain the specific factors required by the purple bacteria, it is safe to assume that the accompanying microflora will synthesize them. It is, therefore, possible that the *Athiorhodaceae*, limited in their natural distribution to localities where growth factors always occur, have lost the property to synthesize one or more of them.

A comparison with the nutrition of the *Thiorhodaceae* is of interest in this connection. The few members of that group which have so far been studied in pure culture do not appear to require any such growth factors. But it must also be remembered that the natural habitat of these bacteria is not restricted to muds and similar places where anaerobic decomposition processes go on, liberating the hydrogen sulfide necessary for growth of the sulfur bacteria. They also occur in sulfur spring waters, and in this environment they are not likely to encounter a supply of organic growth factors. These ecological reflections are, of course, adapted from the ideas so ingeniously propounded by Lwoff (60, 61), as early as 1936, in connection with the parasitic nature of certain bacteria.

The reason for this excursion into the realm of growth factors is that the available evidence rather strongly supports the belief that the yeast extract, etc., found to be necessary in order to secure development of the *Athiorhodaceae*, serves mainly as a source of one or more such substances. Firstly, it has been demonstrated that the quantity of yeast extract needed for full development is very small indeed. Furthermore, several experiments have proved that with low concentrations of yeast extract a maximum development is shown only by cultures in media which contain ammonia-nitrogen as well. These will be discussed presently; let us first return to the problem of the general nutritional requirements.

b. Carbon requirements. In media prepared with the standard inorganic salts, including a sufficient supply of carbon dioxide in the form of sodium bicarbonate, and varying quantities of yeast extract, the development of the non-sulfur purple bacteria is, as would be expected, very nearly proportional to the amount of yeast extract as long as this is used in low concentration. If the complex organic substrate is not in excess of 1 ml liquid yeast autolysate per liter of inorganic medium, the growth is practically negligible. But if to such a culture solution any one of a number of simple organic substances is added, the *Athiorhodaceae* develop profusely. This, then, demonstrates conclusively that such substances can be and are used by these organisms as the main nutrients.

Thousands of cultures of this kind have been set up and examined during the past several years. The results have made possible an interpretation which is

in full agreement with the previous theoretical deductions. Furthermore, it has become clear why different investigators, using different methods for the study of physiological problems of the non-sulfur purple and brown bacteria, have reported such widely divergent conclusions.

In general, it can be stated that the various strains belonging to this group can utilize a considerable number of simple organic compounds. In complete agreement with Gaffron's manometric experiments with cell suspensions, it has been demonstrated that fatty acids represent excellent substrates for growth of the organisms. This, however, appears conclusively only from such experiments in which the culture medium does not contain a large enough amount of peptone or yeast extract to result in dense growth in the absence of a fatty acid salt.

Under such conditions the growth is rigorously proportional to the amount of organic acid present in the medium, as long as the latter is added in small amounts. Table 3 summarizes the results of one representative experiment in which a mineral medium, containing 1 ml per liter of liquid yeast autolysate was used with increasing amounts of sodium acetate. These data show not only the strict proportionality between available acetate and cell yield, but they permit

TABLE 3

*Cell yield of purple bacteria grown in the presence of sodium acetate**

ACETATE CONC. (PER CENT)	0	0.05	0.10	0.20	0.30
μl cells per 10 ml	<0.5	9	18	35	56

* Medium contained 2 ml per liter yeast autolysate; see below.

some further computations which reveal important features of the growth of purple bacteria in such media.

The cell yield was determined by centrifugation for 1 hour; the cells were tightly packed. The dry weight of the sediment is approximately 20%, and from previous chemical determinations it is known that the dry cell material contains 55–56% C and 11–12% N (16). Disregarding the very small amount of organic material contributed by the 0.1% yeast autolysate, which itself does not allow measurable growth in the absence of acetate, the figures of table 3 can then be converted so as to show how much of the carbon, available as acetate, has been converted into cell material (table 4). From the fact that these approximate calculations show that the conversion of acetate-carbon into cell material proceeds with an efficiency of about 70% we must now also conclude that the acetate is used in growing cultures of *Athiorhodaceae* in much the same manner as in anaerobic, illuminated cultures of *Thiorhodaceae*. For the latter organisms, Muller (7) determined by direct chemical measurements a conversion of 63–82% of acetate-carbon into cell substance. The efficiency is, furthermore, of the same order of magnitude as that observed by Gaffron with cell suspensions under conditions where growth was excluded. Such determinations (1, 5, 8) have demonstrated that around 80% of the acetate-carbon used was converted into assimilatory products.

This magnitude of the efficiency is, of course, possible only on account of the photosynthetic nature of the metabolic process. Considering that the primary assimilatory product must subsequently be converted into the multitude of cell constituents, it is understandable that during the secondary conversions losses, mainly if not exclusively in the form of carbon dioxide, occur.

If one continues to increase the acetate concentration above 0.3% the growth of purple bacteria soon ceases to increase in direct proportion to the amount of substrate. It seems that this is due to the fact that a maximum population is reached which cannot, under ordinary circumstances, be surpassed. Here again, a surprisingly close agreement is revealed between Gaffron's experimental results and my own. Using yeast extract media, corresponding in my experience to about 10% by volume of yeast autolysate, enriched with sodium citrate and potassium butyrate, and an inoculum of an impure culture of purple bacteria, Gaffron reports a yield of about 1 gram dry bacteria per liter (1). The same amounts of cell material were obtained with pure cultures of *Spirillum rubrum* in yeast extract-glycerin media (62), and, later, with various strains of *Athiorhodaceae* in a mineral medium with yeast autolysate and sodium malate (unpubl.).

TABLE 4

Relationship of carbon in cell material and in medium

CONC. OF NaC₂H₃O₂, PER CENT	0.05	0.10	0.20	0.30
Cell material from 10 ml				
a. mg dry weight	1.8	3.6	7.0	11.2
b. mg carbon	1.0	2.0	3.9	6.25
Acetate available in 10 ml as mg carbon	1.44	2.9	5.8	8.7
Per cent conversion	70	69	67	72

From these data one may infer that the yield of 56 μl of cells per 10 ml of medium, obtained in the above-discussed experiments with 0.3% acetate, approaches quite closely the maximum yields obtained with what at first sight would seem to be vastly more satisfactory media.

These results also explain why Czurda and Maresch (20) failed to observe better growth of purple bacteria upon the addition of fatty acids to their medium, since the organisms can develop to the maximum extent in ordinary yeast extract or 1% peptone media without any further additions. Hence it is clear that experiments in which such concentrated solutions of complex materials are used cannot be expected to yield information concerning the nutritional value of added substances. It still remains possible that even in the experiments of Czurda and Maresch the fatty acids were utilized by the bacteria, leaving more of the peptone or yeast extract untouched. But this could have been decided only on the basis of chemical determinations at the end of the incubation period.

What has so far been remarked concerning the growth of the *Athiorhodaceae* in the presence of acetate can be applied, with a few provisions, to development with other fatty acids as well. However, two factors must be borne in mind in any attempt to extrapolate the results of the acetate cultures. The first is that

the higher fatty acids are toxic in relatively low concentrations. Since the toxicity is primarily due to undissociated acid molecules, their inhibitory effect is enhanced in acid media (See also (5, 16)). Whereas concentrations of sodium acetate of 0.3–0.5% can be tolerated by the bacteria in a neutral medium, a considerably lower concentration of the higher fatty acids must be used in order to insure growth. For some strains even 0.05% of the valeric acids and higher members of the series does not permit growth; other strains can tolerate these substances up to 0.1%.

This toxic effect of the higher fatty acids must be held responsible for some anomalies in Nakamura's publication (57). From his experiments on photosynthesis by *Rhodobacillus palustris* in the presence of various fatty acids it would appear that *n*-caproic acid and the higher homologs cannot be utilized. The same results were obtained in respiration experiments with the Thunberg technique. In the former series the final substrate concentration in the suspension was M/120; in the latter it appears to have been as high as M/40. Gaffron, using final concentrations of M/400, had found that substrates which were ineffective in Nakamura's experiments, were readily metabolized (1).

The above-mentioned results were secured in experiments with dense suspensions of non-proliferating bacteria. But also in culture experiments, it has been established that growth of many, if not all, members of the *Athiorhodaceae* is possible in media with heptylic, caprylic, and pelargonic acids, provided these substances are used in concentrations of not over 0.03% (M/500–M/1000). Higher members of the fatty acid series were not investigated because of their relative insolubility. From Gaffron's results one might expect growth to occur also in their presence, at least up to stearic acid.

A priori one might expect that the toxicity of a fatty acid can be represented as a simple function of its concentration and the pH of the medium. Some culture experiments, designed to establish this relationship for a number of fatty acids on a quantitative basis have been conducted. But the results cannot yet lay claim to the required accuracy for making them significant. This is in part due to the fact that, as soon as metabolism starts, the alkalinity of the culture solution increases as a result of the simultaneous assimilation of carbon dioxide and the practically complete conversion of the anion or acid molecule into cell substance with the cations remaining in solution. Thus a rise of one pH unit, even in media containing 0.3% (M/30) phosphate buffer, is of common occurrence if the initial pH is below 6.8. Added to this is the difficulty that media on the acid side of neutrality in glass-stoppered bottles tend to lose carbon dioxide. These difficulties can, of course, be overcome by such devices as constant equilibration with gas mixtures containing the requisite amount of carbon dioxide; with such refined methods the experiments have, however, not yet been carried out. The preliminary results obtained so far corroborate the expectation that the lower the pH, the lower is also the total fatty acid concentration which will still permit development. That this is not due to a direct pH effect on the organisms was clearly demonstrated by the inclusion of cultures with utilizable substrates of a non-acidic nature. Such cultures have shown that many of the

non-sulfur purple bacteria can grow satisfactorily in media at pII 6.0, *i.e.* far below that which in combination with low concentrations of fatty acids inhibits development.

For growth experiments with higher fatty acids one is thus compelled to use increasingly low substrate concentrations for the higher homologs. Ordinarily this might imply a decrease in cell yield to such an extent that it would become difficult to evaluate the difference in growth in the media with and without added substrate. However, the metabolism of the purple bacteria in the presence of higher fatty acids results not only in a complete conversion of the organic substrate into cell substance, but is accompanied by the actual assimilation of carbon dioxide, as shown by the experiments of Gaffron and others (1, 5, 7, 16). The carbon dioxide uptake is proportional to the length of the carbon chain of the fatty acid, so that for every additional CH_2 group about 0.4 mol of CO_2 is fixed. This means that a maximum yield of bacterial cells can be obtained with far smaller concentrations of the higher fatty acids than with acetate. Again, the

TABLE 5

Relation between yield of cell material and fatty acid used as substrate

FATTY ACID	MOL. WT. OF Na-SALT	MG CARBON PER MILLIMOL	CO_2-UPTAKE PER MILLIMOL		MG CARBON IN CELL MATERIAL FROM 1 GM SUBSTRATE
			millimol	mg Carbon	
Formic acid	6S	12	−0.6	−7.2	75
Acetic acid	S2	24	−0.2	−2.4	230
Propionic acid	96	36	0.3	3.6	350
Butyric acid	110	4S	0.65	7.8	435
Valeric acid	124	60	1.0	12.0	500
Caproic acid	13S	72	1.4	16.S	550
Heptylic acid	152	S4	1.7	20.4	600
Caprylic acid	166	96	2.0	24.0	635
Pelargonic acid	1S0	10S	2.4	2S.S	665

proportionality of CO_2-uptake and length of carbon chain was first established by manometric measurements of the metabolism of cell suspensions of *Athiorhodaceae*. These results have now been fully confirmed by growth experiments. The average cell yield per gram of sodium salt of various fatty acids is presented in table 5. It will be seen that the amount of cell material obtainable with, *e.g.*, pelargonic acid is almost three times as large as with acetic acid per gram of substrate. The latter substrate yields a maximum crop when used in concentrations of about 0.3%, amounting to around 55–60 μl of cells per 10 ml of medium. Since as much as 25 μl of bacteria may be produced in media containing only 0.05% sodium pelargonate, an evaluation of the growth in media containing very small amounts of the higher fatty acids as the chief substrate presents no difficulties.

From a theoretical consideration of the conditions in enrichment cultures of *Athiorhodaceae* it was deduced that various substances resulting from the primary decompositions of the chemically complex plant and animal materials might

logically be expected to serve as the substrates for the purple bacteria. The experiments with the various fatty acids have thus fully confirmed the expectations for this one group of substances. But the same holds true for a variety of other organic compounds which normally occur as products of the anaerobic decomposition of complex substrates. This has been shown by using the same general technique as was employed with the fatty acids, viz., the inoculation of a mineral medium containing a small amount of yeast autolysate as a source of growth factors, and various simple carbon compounds as the chief substrate, with pure cultures of non-sulphur purple bacteria. Since growth at the expense of the yeast autolysate alone is negligible, the availability of the organic compounds tested can be judged by comparing the development of the strains in the different media. In this manner it has been found that also various unsaturated acids, hydroxy acids, dibasic acids, etc., as well as a number of amino acids can be used instead of fatty acids.

Of particular interest are the results obtained with non-acidic compounds. Gaffron had concluded from his manometric experiments that only substances with a carboxyl group can be used as assimilation substrates by the *Athiorhodaceae*. He wrote in 1935: "Das Vorhandensein einer Carboxylgruppe ist Grundbedingung dafür, dass ein Körper als Substrat der Assimilation dienen kann" (5, p. 308). Since, however, alcohols undoubtedly occur among the primary decomposition products of the complex materials used in enrichment cultures, the previous ecological considerations would lead one to expect that these simple substances could be used at least by some of the non-sulfur purple bacteria. And this all the more because many microörganisms, capable of growing at the expense of fatty acids, are known to be endowed with the ability to use the corresponding alcohols as well.

Foster (6) was the first to clarify the situation. He showed, both by growth experiments and by manometric measurements, that certain strains of *Athiorhodaceae* are indeed able to utilize several simple alcohols, both primary and secondary. The former are usually converted entirely into cell material, whereas the latter are oxidized to the corresponding ketones with a concomitant reduction of carbon dioxide. His experiments also indicated the reasons for Gaffron's failure to observe the utilization of alcohols by the purple bacteria, the rate of alcohol oxidation being considerably lower than that of the decomposition of fatty acids, even under the best conditions. Furthermore, the latter are realized only when cells are used which have been grown with alcohols as the chief organic compounds of the medium. Foster found that cells from cultures in the more common, alcohol-free media will not metabolize alcohols at a measurable rate except after a prolonged period of "adaptation," a process which requires incubation of the organisms in the presence of alcohol for many hours. (Personal communication). Consequently it is evident why Gaffron, using concentrated yeast extract media for growing the bacteria, and measuring their metabolism over short periods of time only, reached his conclusion.

Apart from the simple alcohols, studied by Foster, also polyvalent alcohols (glycerol, mannitol, sorbitol) and carbohydrates can be used by some repre-

sentatives of the *Athiorhodaceae*. But it should be emphasized that not all members of this group have the same physiological characteristics. While a more systematic survey of the results obtained will be presented further on, it may here be pointed out that there exist definite correlations in this respect between the morphological features of the various representatives and their ability to grow in the presence of special organic compounds. A few examples may serve to illustrate this point.

It may be recalled that the experiments of Molisch had indicated that glycerol and certain carbohydrates should be considered as the best substrates for the non-sulfur purple bacteria in the presence of peptone. A complete survey of the nutritional requirements of the large number of pure cultures has convinced me that this is not the case. Although there are types which can utilize these substances in the mineral medium with 0.1% yeast extract, many strains fail to grow in the presence of glycerol, and a large number appear unable to use carbohydrates. Still fewer develop in media in which mannitol or sorbitol are the major carbon sources.

Most surprising, especially in view of the previously reported results obtained by other investigators with dextrin and inulin, was the observation that the numerous strains which correspond closely to Molisch's description of *Rhodobacillus palustris* appear unable to use the simple hexoses, glucose, mannose, and fructose. It must, however, be realized that the polysaccharides recommended by Molisch are usually very far from chemically pure. It is, therefore, logical to believe that the satisfactory growth resulting from the use of the latter substances must be ascribed to the presence of impurities.

This conclusion is supported by two independent facts. Firstly, Nakamura (57) found that of seven carbohydrates, tested as substrates for *Rhodobacillus palustris*, only dextrin appeared to be used. At present there is less reason than ever to believe that polysaccharides can be used without undergoing a preliminary depolymerisation; the putative experimental support for the opposite contention has, in each case that has been carefully examined, been shown to rest on a misinterpretation or faulty technique.[2] Nakamura's, as well as Molisch's results strongly indicate, therefore, that the impurities in the dextrin used caused the observed effects.

The second line of evidence for this interpretation is furnished by the results obtained with two different fructose preparations. With the first I invariably observed good growth of all strains of purple bacteria, while several of them failed to develop in the corresponding glucose media. A second, better grade batch of fructose, as also the product obtained from the first by repeated recrystallization, yielded negative results wherever glucose did. These experiments demonstrate conclusively that impurities in commercial carbohydrate preparations do occasionally cause anomalous results.

In connection with the established inability of *Rhodobacillus palustris* to utilize sugars special mention must be made of Schneider's claim (22) that this organism does not grow in peptone-glucose media. From my own experiments it has

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[2] See especially Kluyver and Custers (63), and Stanier (64).

appeared that development occurs in peptone solutions without sugar, and, furthermore, I had never noticed an inhibitory effect of glucose on my strains. A repetition of the experiments with Schneider's isolates confirmed his results. But the interpretation is not that glucose itself is toxic to some strains of *Rhodobacillus palustris*. The cultures, kindly furnished by Dr. Schneider, were contaminated with lactic acid bacteria, and the rapid development of these organisms in sugar-containing media caused an increase in acidity sufficient to prevent the growth of the purple bacteria. Also the production of acid in glycerin media, reported by Schneider, is not due to the metabolic activities of *Rhodobacillus palustris*, but to contaminating microörganisms.

Having thus disposed of the conflicting reports on the effects of sugars on the non-sulfur purple bacteria, it may be concluded that these substances can be used to differentiate between various representatives of the group. But not only the carbohydrates and related substances are valuable for this purpose; equally characteristic differences in the behavior of the various strains are exhibited with respect to other substrates, such as propionate, tartrate, citrate, certain amino acids, and alcohols. Other compounds, especially succinic and glutaric acids, although utilizable by all strains, yield only meager cultures in some cases, sharply contrasted with the profuse development of other strains in the same medium. The only substrates which appear to be equally satisfactory for all strains are acetate, butyrate, crotonate, lactate, malate, and a few others.

These results necessitate a complete revision of the prevalent ideas concerning the carbon nutrition of the *Athiorhodaceae*. At the same time they can and will be used for a more complete characterization of the different species of this group, and ultimately, have served the purpose of devising enrichment culture methods for several of the species that can be recognized at present.

c. Nitrogen requirements; growth factors; minerals. Since the foregoing experiments were made possible only after it was found that a low concentration of yeast autolysate could be substituted for the commonly used 1% peptone or yeast extract media, a brief discussion of the effect of different concentrations of the latter material on the development of the purple bacteria is in order. The experiments carried out in connection with the problem have been conducted mostly with media in which acetate was used as the chief substrate. Occasionally, however, they have been repeated with different compounds, and, because the results have always been in complete agreement with those of the former series, they need not be dealt with separately. I believe that it is safe to assume that more extensive future investigations will not materially change the fundamental aspects of the nutrient requirements which have been developed with the aid of acetate cultures.

As usual, the general results will be illustrated by a representative experiment.

To a sterile mineral medium, prepared with distilled water containing 1 gram $(NH_4)_2SO_4$ and 0.2 g $MgCl_2$ per liter, were added measured amounts of sterile Na_2S and $NaHCO_3$ solutions, the latter sterilized by filtration through Seitz filters under pressure, so that the final concentrations were 0.005% and 0.3% respectively. The reaction was adjusted to pH 7.0 with sterile H_3PO_4 solution, after which 30 ml of a M/1.5 phosphate buffer (pH 7.0) were added per liter of medium. This basal medium was enriched with varying amounts of

sterile yeast autolysate and sodium acetate, inoculated with a pure culture of a *Rhodospirillum* strain, and dispensed aseptically into sterile, glass-stoppered bottles, the latter being completely filled and stoppered. The cultures were incubated in a light cabinet

TABLE 6

Development of Rhodospirillum sp. in the presence of different amounts of yeast autolysate and sodium acetate

EXPT. NO.	YEAST AUTOLYSATE ML PER LITER	SODIUM ACETATE G PER LITER	CELL VOLUME IN μL PER 10 ML
1	0	0	0
2	0.1	0	0
3	0.2	0	0
4	0.5	0	0
5	1.0	0	0.5
6	2.0	0	0.5
7	3.0	0	0.5
8	0	0.5	0
9	0.1	0.5	4
10	0.2	0.5	6
11	0.5	0.5	8
12	1.0	0.5	9
13	2.0	0.5	8
14	3.0	0.5	9
15	0	1.0	0
16	0.1	1.0	7
17	0.2	1.0	16
1S	0.5	1.0	17
19	1.0	1.0	18
20	2.0	1.0	18
21	3.0	1.0	19
22	0	2.0	0
23	0.1	2.0	10
24	0.2	2.0	20
25	0.5	2.0	33
26	1.0	2.0	36
27	2.0	2.0	35
2S	3.0	2.0	39
29	0	3.0	0
30	0.1	3.0	10
31	0.2	3.0	21
32	0.5	3.0	*
33	1.0	3.0	57
34	2.0	3.0	56
35	3.0	3.0	5S

* Not determined.

with constant illumination at a temperature of 30°C. After they had reached full development, the contents of the bottles were thoroughly mixed, and aliquots centrifuged for the determination of the total cell volume. The results are presented in table 6.

It is clear that it is not only the acetate concentration which determines the final cell yield, but that the latter is profoundly influenced by the amount of yeast autolysate as well. Although it is true that the development is proportional to the amount of acetate in the presence of enough yeast autolysate, nevertheless, this proportionality is reached only with increasingly large amounts of the latter substrate. In figure 17 the interrelations are shown graphically.

Evidently, the medium with the lower yeast extract concentrations is deficient in some material needed for development; with 0.1 ml per l the maximum cell yield amounts to 10 μl per 10 ml. A two-fold increase in the amount of yeast extract will allow of a corresponding rise in the number of cells, provided, of
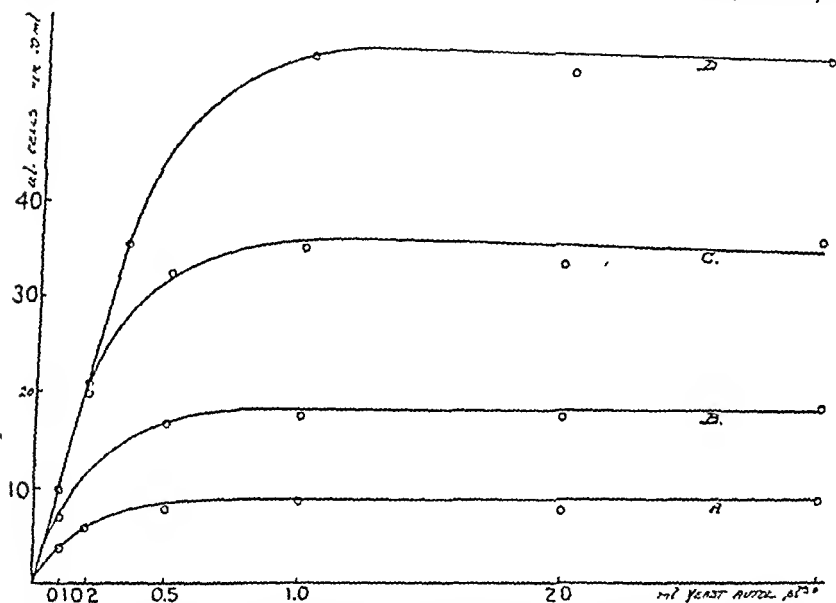
FIG. 17. Relation between cell yield and yeast extract concentration in cultures of *Rhodospirillum rubrum*, Strain No. 6, in basal medium with Na-acetate.
Curve A—0.05% acetate; Curve B—0.10% acetate; Curve C—0.20% acetate; Curve D—0.30% acetate.

course, the medium contains enough acetate. With 1 ml or more yeast autolysate per liter the growth appears to be limited by the acetate.

About the nature of the special material which is supplied by the yeast extract not much can yet be said. It is, however, certain that the autolysate does not supply the bacteria with more than a small fraction of their nitrogen requirements. This follows from the simple computation of the total quantity of nitrogen present in the yeast extract and in the bacteria in densely populated cultures. The extract contains around 1% total nitrogen; the nitrogen content of the organisms is about 11–12% of the dry weight (16). Hence 1 ml of yeast autolysate contains about 10 mg total nitrogen. Yet this small amount is adequate to produce, in the presence of enough acetate, a quantity of cell material

as large as 5.5 ml, representing a dry weight of approximately 1 gram, with 110–120 mg organic nitrogen. Even if all the nitrogen of the yeast extract were utilizable by the bacteria for conversion into cell materials, the total supply would be not more than one tenth of the amount required. It is, therefore, necessary to conclude that the ammonium salts in the medium are used as the main nitrogen source.

This has been further supported by experiments in which a standard medium with 1 ml of yeast autolysate per liter and 0.2–0.3% acetate was used, containing varying amounts of $(NH_4)_2SO_4$. They have shown that the last-mentioned substance, if present in very low concentrations, can become a limiting factor for growth. Above 0.03% $(NH_4)_2SO_4$ no effect is observable; at lower concentrations the development of the bacteria remains below the possible maximum in proportion to the amount of $(NH_4)_2SO_4$ supplied.

So far I have not carried out more than a few preliminary experiments in an attempt to identify the substance or substances in the yeast extract which are necessary for growth of the *Athiorhodaceae*. The data are not yet interpretable in terms of distinct chemical entities. It is, nevertheless, clear from the experimental results that different members of this group of organisms appear to have different requirements. Generally, growth is possible with as little as 1 ml of yeast autolysate per liter in the presence of a suitable, nitrogen-free organic substrate. But a number of strains show a vastly increased rate of development, as well as greater final yields of organisms with considerably higher concentrations.

An interesting effect pertaining to the mineral nutrition of the organisms may also be mentioned at this point. The medium usually employed contains only the elements H, O, C, N, P, S, K, Na, and Mg in appreciable quantities. Special precautions for avoiding the presence of other elements were never taken, and in view of the necessary addition of yeast extract it is reasonable to assume that many more elements were, indeed, present, and in sufficient quantities to meet the requirements. One of these plays an important role at least in the nutrition of the purple spirilla.

During his investigations on the oxidation of alcohols by members of the *Athiorhodaceae*, Foster made the observation that *Rhodospirillum* strains often develop as a flocculent precipitate instead of producing the more typical homogeneous, deep-red cultures. This abnormal mode of growth he traced to a lack of sufficient calcium in the medium; the addition of 0.01% $CaCl_2$ sufficed to prevent the formation of the tangled masses of sprilla which constitute the precipitate. (Personal communication.) Such a relatively high calcium concentration does not, however, influence the amount of growth, the latter being essentially the same with and without added calcium salt. It is thus likely that its peculiar effect must be attributed to special ionic interrelations or balances, determining the behavior of surfaces (See, in this connection, Heilbrunn, (67)).

As long as the chemical nature of the essential substances supplied by the yeast extract is not known, it is clearly impossible to study effectively the exact mineral requirements of the *Athiorhodaceae*, and the possible role of "trace elements" in their nutrition. Although the bacteria generally do not lend themselves particularly well to an experimental approach to this problem, the abun-

dant development of the non-sulfur purple bacteria in media with very low concentrations of the different ingredients may render these organisms valuable for the study of mineral nutrition once it becomes possible to add the as yet unknown factors in a chemically pure form.

d. Inorganic substances as substrates for photosynthesis and growth of the non-sulfur purple bacteria. The foregoing sections have shown that a large variety of simple organic compounds can be used by the representatives of the non-sulfur purple bacteria for photosynthesis as well as for growth. But those are not the only possible substrates for organisms of this group.

In 1933 Gaffron published the important observation that his (impure) cultures of *Rhodobacillus* could also bring about an oxidation of hydrogen sulfide, coupled with a photochemical reduction of carbon dioxide (1). Similar results were later obtained by Nakamura (57) with pure cultures. And when, in 1934, Roelofsen (54, 68) established that molecular hydrogen can be used for photosynthesis by *Thiorhodaceae*, Gaffron soon afterwards reported the same reaction for representatives of the *Athiorhodaceae* (5). Since then various investigators have corroborated the occurrence of these reactions (16, 32, 57, 69–73). It must, however, be pointed out that not all strains of this group are characterized by the ability to use sulfide or hydrogen; in 1930 I had conducted a number of experiments on photosynthesis in the presence of sulfide and of hydrogen with *Rhodospirillum* strains, obtaining completely negative results.

The above-mentioned investigations had all been carried out with the aid of the manometric technique, and hence with cells previously grown in organic media. It is, however, obvious that those purple bacteria which can utilize oxidizable inorganic substances for photosynthesis should also be able to grow, in the proper media, chiefly on the basis of these reactions. This has been verified by numerous culture experiments.

The first of these were conducted in 1930, in an attempt to grow *Athiorhodaceae* in sulfide media. By using an inorganic medium with very small amounts of peptone and various sulfide concentrations it was demonstrated that the presence of sulfide results in increased growth of the organisms in anaerobic, *illuminated* cultures. It was also observed that, at the end of an experiment, the sulfide had become oxidized, mainly to free sulfur. This result was reported in 1935 (52) and attention drawn to the apparent discrepancy with Gaffron's observations from which a complete oxidation of sulfide to sulfate could be inferred. It is now clear that the incomplete oxidation in the culture experiments was due to the use of media which allowed only a very scanty development. Since the further oxidation of elementary sulfur proceeds extremely slowly by the small number of cells present—the development being limited by the lack of sufficient growth factors—it may easily be overlooked! In manometric experiments, on the other hand, where a large number of organisms is used along with a very small quantity of sulfide, a complete oxidation can be readily demonstrated. From the culture experiments it follows, however, that the oxidation of sulfide to sulfur takes place preferentially to a further oxidation of the latter. This is also substantiated by the statement of Nakamura (57) that *Rhodobacillus* cells from a sulfide-containing medium are covered with sulfur globules.

All sulfur purple bacteria can utilize thiosulfate as well as sulfide. Hence it was reasonable to expect that those *Athiorhodaceae* capable of oxidizing sulfide could also attack thiosulfate, and even grow at the expense of this oxidation. This has been tested experimentally, and the results have been unambiguous. In a medium with only a small amount of yeast extract, which by itself yields no more than a barely perceptible growth, several strains produce good cultures upon the addition of 0.1–0.2% $Na_2S_2O_3$. That the latter is converted into sulfate has been shown by chemical determinations of the sulfate content of blanks and inoculated media. Without yeast extract or other complex materials growth has not been observed in thiosulfate solutions.

Under similar conditions the development of certain strains of non-sulfur purple bacteria has been shown to proceed at the expense of molecular hydrogen.

Thus the experimental evidence, demonstrating the ability of *Athiorhodaceae* species to grow by virtue of their utilization of sulfide, thiosulfate, and molecular hydrogen, adds still another argument in favor of the previous ecological considerations. It has now been shown that the various products resulting from the primary decomposition of plant and animal remains can, one and all, serve as major substrates for the development of the non-sulfur purple bacteria.

At this point I regret to announce that so far one group of substances, the lower amines, has been omitted from the list of substrates investigated. This omission is particularly deplorable because these compounds will undoubtedly be formed during the anaerobic decomposition of proteinaceous materials. It is therefore *a priori* to be expected that the amines, too, can be used for photosynthesis and growth by some members of the group. During his extensive studies on the aerobic decomposition of various groups of carbon compounds den Dooren de Jong (74, 75) found the lower aliphatic amines to be attacked principally by a small group of apparently highly specialized bacteria, the *Protaminobacter* species.[3] In view of the existence of this specialized group of aerobic bacteria it is quite conceivable that also among the purple bacteria typical "specialists" for the oxidation of these amines would be encountered. It is hoped that this gap in our present knowledge will soon be filled.

e. The nutrition of the non-sulfur purple bacteria in the dark. While the foregoing discussion of the physiological characteristics of the non-sulfur purple bacteria has dealt chiefly with the nutrient requirements of the organisms when grown under anaerobic conditions and in the light, a few remarks should be added concerning the development under aerobic conditions, and in the dark.

On a number of occasions it has already been pointed out that those strains which can develop in the presence of air, can also be grown in the dark, but only aerobically. The media which will permit development are essentially the same as those in which growth can occur in the light. It must, however, be remembered that in the latter case carbon dioxide appears to be used as the only final hydrogen acceptor for the oxidation of the substrate. Furthermore, this carbon dioxide reduction results exclusively in the production of cell materials. Hence

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[3] den Dooren de Jong proposed the generic name *Protaminobacter* for these organisms. Janke (76) has, however, pointed out that this is a misnomer, and substituted the designation *primoraminophagous* microbes for the members of den Dooren de Jong's genus.

the amount of cell material produced is of the same order of magnitude as the amount of substrate decomposed. This is not the case if the organisms must depend on oxygen as an ultimate hydrogen acceptor, i.e., when they develop in the dark. Under these circumstances a large proportion of the organic substrate is decomposed with the liberation of CO_2—and NH_3 if the substrate contains amino acids which can be utilized—so that the yield of cell material from the same amount of substrate is naturally much smaller in aerobic, dark cultures than in illuminated anaerobic ones.

This difference in yield becomes particularly striking if an inorganic oxidizable substrate, such as hydrogen or thiosulfate is used. It is evident, and in the next section this point will be elaborated, that under these circumstances the growth of the purple bacteria becomes virtually autotrophic. Now, it is well known that the "efficiency" of autotrophic, non-photosynthetic bacteria, as reflected in the relation between the amounts of cell material formed and of the substrate oxidized, is usually quite low. This means that most of the available hydrogen is ultimately transferred to oxygen, and that only a small fraction is used for the production of cell substance from carbon dioxide. The elimination of the substrate oxidation with molecular oxygen by culturing the organisms anaerobically in the light must, therefore, of necessity increase the cell yield many-fold.

Nevertheless, from the point of view of the physiological properties of the purple bacteria the important point is not the crop of organisms obtainable under aerobic and anaerobic conditions, but the fact that any medium which will allow the development of the *Athiorhodaceae* by photosynthesis can also serve as a satisfactory substrate for aerobic growth in the dark.

For the ecology of the purple bacteria this fact has little or no importance. While one can thereby understand how the purple bacteria are able to live in nature during periods of darkness, it should by no means be interpreted to mean that in nature these organisms will ever be found thriving under competitive conditions when light is not periodically available to them. The ability to grow aerobically in the absence of light is a physiological potentiality, demonstrable with pure cultures, but one without ecological significance.

Special emphasis should be placed on the fact that none of the *Athiorhodaceae* thus far studied develops in the dark in the absence of oxygen. This is in complete agreement with the observations of Gaffron (1), demonstrating that suspensions of *Rhodovibrio* under these conditions exhibit no measurable metabolic activities, both in the absence and in the presence of a suitable substrate. Later studies (5) have shown, it is true, that suspensions of other members of the group may produce acidic substances when incubated anaerobically in the dark. But the small magnitude of this metabolism on the one hand, and the failure to influence its extent by the addition of substrates, indicate that the phenomenon cannot be considered as comparable with those fermentation processes which enable certain types of microörganisms to develop in the absence of air.

Both Gaffron (5) and Nakamura (57) have found that *Athiorhodaceae* can carry out a nitrate reduction. While the former showed this process to occur in the light, where it is comparable with the photochemical nitrate reduction

observed by Warburg with *Chlorella*, the latter demonstrated that it can also take place in the dark. This makes it possible that non-sulfur purple bacteria, like some common aerobic bacteria, might be grown anaerobically in the dark in nitrate-containing media. Conclusive experiments in this respect have not yet been carried out. But even if this were the case, it would not by any means invalidate the conclusion that the metabolism of the *Athiorhodaceae* is primarily oxidative.

III. A brief characterization of the nutritional physiology of the Athiorhodaceae

a. General aspects. Recapitulating, we may now conclude that the various angles of the nutrition of the non-sulfur purple bacteria, presented in the previous sections, have furnished ecological as well as direct experimental evidence which permits a relatively simple and unified concept of their general physiology.

Like most representatives of the *Pseudomonadaceae*, which they morphologically resemble, the purple bacteria carry out a typically oxidative metabolism. A great variety of simple organic substances—alcohols, fatty acids, hydroxy- and dibasic acids, etc.—can be used as substrates, and these are oxidized during metabolism with the use of an extraneous hydrogen acceptor. Although oxygen is the most common one, other acceptors, notably methylene blue and nitrate (57) can be substituted for it.

Furthermore, the oxidation of organic substances by ordinary aerobic microorganisms results in the production of carbon dioxide, coupled with a "primary assimilation" in the sense of Barker (77), in which a considerable amount of the substrate-carbon is converted into cell material. In a number of carefully studied examples the extent of this assimilation has been shown to be of a high order of magnitude; a conversion of one-half or even two-thirds of the substrate-carbon into assimilation products is common (77–83; 48). The same phenomenon has been observed with cultures of *Athiorhodaceae* oxidizing acetate in the dark in the presence of oxygen (8); even quantitatively the results are in agreement with those obtained with colorless algae, yeasts, *Pseudomonas* and *Spirillum* species.

Again, just as various members of the *Pseudomonadaceae* are capable of oxidizing inorganic compounds, such as thiosulfate and molecular hydrogen, as well as organic substrates, so do certain members of the non-sulfur purple bacteria possess this property.

All these similarities justify the characterization of the metabolism of this group of organisms as typically oxidative, and as essentially similar to that of other oxidative organisms. But in spite of the fact that the above derivation seems eminently logical, and the final conclusion well-nigh unavoidable, this characterisation is, nevertheless, unsatisfactory because it does not include the most outstanding feature of the physiology of the purple bacteria.

The reason for this situation is not hard to discover. The designation "oxidative" of necessity covers only one side of the metabolic activities; it describes the fate of the oxidizable substrate, but not the nature of the final oxidant. Biological oxidations share with the purely chemical ones the requirement that they must be accompanied by concomitant

reductions. And, although the oxidizability of a substance by a variety of oxidizing agents has long been recognized in chemical reactions, the term "oxidative" in biological processes has often been unconsciously construed to imply that the oxidant is molecular oxygen. It is true that this restriction was eliminated by Wieland's demonstrations that other substances, such as quinone and methylene blue, could function as hydrogen acceptors (i.e. oxidizing agents); yet the tendency has been to consider such systems more as interesting potentialities, induced by artificial laboratory conditions, than as biologically important realities.

Yet, it is especially among the microörganisms that one meets with cases in which the implication of a typically "oxidative" metabolism should not be that molecular oxygen enters into the reaction as final hydrogen acceptor. The most clear-cut examples are the processes of nitrate reduction, of sulfate reduction, and of carbonate reduction. The first, it is true, is ordinarily carried out by organisms which are essentially aerobic, and which can, therefore, normally use molecular oxygen for the oxidation of the substrate. It is thus possible to consider this process as an "aberrant" type of oxidative metabolism, imposed by the environmental conditions, and on a par with the reduction of methylene blue under anaerobic conditions. This line of reasoning cannot, however, be applied to the other two processes. The sulfate-reducing bacteria are anaerobes; they cannot live in the presence of air. At the same time, the fate of the oxidizable substrate is in no way different from that which typifies its degradation under the influence of an aerobic, oxidative organism. The important distinction is that, as far as is known, the sulfate-reducing bacteria can use only one specific group of substances, viz. the oxidized sulfur compounds sulfate, sulfite, thiosulfate, etc., as the ultimate hydrogen acceptors. The same appears to be true for the bacteria causing the methane fermentation; the only known acceptor for these organisms is carbonate or carbon dioxide (See Barker (84–87)). Consequently it appears that the term "oxidative metabolism" comprises a number of processes, similar or identical with respect to the fate of the oxidizable substrate, but characteristically different and readily distinguishable on account of the final oxidizing agent.

By taking this more comprehensive view of oxidative metabolism into consideration, it becomes possible to characterize the physiological properties of the purple bacteria more adequately. This group of organisms possesses the ability, unique among the bacteria, of carrying out an oxidative degradation of various substrates with carbon dioxide as the only final hydrogen acceptor, *but dependent upon proper illumination*. What this implies concerning our concepts of the process of photosynthesis has been discussed in more detail elsewhere (8).

Of great importance for an evaluation of the relative physiological importance of the photosynthetic and the "dark" oxidation of a substrate are some experiments of which an account has been rendered previously (8). *It has repeatedly* been pointed out that the *Athiorhodaceae* are capable of oxidizing one and the same substance either in the dark or in the light. In the former case oxygen is normally used as the ultimate oxygen acceptor, while in the latter the oxidation can proceed under anaerobic conditions because here carbon dioxide fulfills this function. Now it has been shown conclusively (8) that suspensions of nonsulfur purple bacteria, in the presence of oxygen, carbon dioxide, and an appropriate substrate, fail to consume oxygen if the suspensions are illuminated. In spite of the presence of oxygen, the carbon dioxide utilization is exactly the same as in comparable experiments carried out under anaerobic conditions. Consequently in illuminated cultures the photosynthetic mechanism appears to be the only functional one, and the complete suppression of oxygen consumption by

illumination demonstrates convincingly that the purple bacteria must be considered primarily as photosynthetic organisms.

b. *Thio-* and *Athiorhodaceae.* Physiologically, the non-sulfur purple bacteria are thus related on the one hand to the purple and green sulfur bacteria by virtue of the photosynthetic nature of their metabolism, and on the other to the various types of non-photosynthetic organisms with a typically oxidative metabolism. With regard to the first-mentioned relationship, which has already been discussed to some extent in connection with the delimitation of the group of *Athiorhodaceae*, a few additional remarks are here in order.

While both *Thio-* and *Athiorhodaceae* can carry out a photosynthetic process in the presence of various oxidizable substances, a satisfactory differentiation seemed possible on the basis of the ecologically important fact that in nature the sulfur purple bacteria appear to be restricted to those localities where sulfide is present, while the other group develops primarily in organic media. Now that it has been shown, however, that organisms which have for many years been considered as typical *Athiorhodaceae* are also capable of utilizing oxidizable sulfur compounds instead of organic substances, this distinction becomes much more difficult.

An additional feature has been brought out by the investigation of the nutrition of the non-sulfur purple bacteria which can be used to separate the two groups. This is the recognition that the various members of the *Athiorhodaceae* require special, as yet unknown, growth factors of an organic nature. Whereas, therefore, *Thiorhodaceae* may develop in strictly inorganic media, this is not possible for representatives of the other group. It is well to remember, however, that only a very few of the typical sulfur purple bacteria have so far been obtained and studied in pure culture, so that it is conceivable that growth of some types may yet be shown to depend upon the presence of specific organic materials. With this in mind I have, during the past several years, made a number of attempts to culture some of the large *Thiorhodaceae*, particularly *Chromatium okenii*, by using sulfide-containing media enriched with complex organic materials (yeast extract, etc.), but the results have not been encouraging. Also, one must admit the possibility that certain species of *Athiorhodaceae* will be discovered which can develop in the absence of special organic growth-factors. If such organisms were simultaneously endowed with the ability to oxidize sulfur compounds, they would physiologically become indistinguishable from the *Thiorhodaceae* on the basis of our present knowledge. And the small pseudomonads, described in 1931 as typical sulfur purple bacteria (3), may be regarded with some justification as a case in point. This is all the more true since I have obtained enrichment cultures of these organisms with organic media inoculated with marine mud samples.

Thus the oft-repeated complaint of the systematist that nature knows of no sharp distinctions, and thereby renders his attempts arbitrary, may again be reiterated. While there is every reason to favor a subdivision of the purple bacteria into two separate groups, and while it is clear enough that this can readily be achieved if we consider only the most characteristic representatives

of the two entities, it remains a difficult task to select and define the criteria which appear to be the most logical and useful. We shall face this problem in a later section on the classification of the *Athiorhodaceae*.

c. Auto- and heterotrophic bacteria. The physiological similarities between the photosynthetic non-sulfur purple and brown bacteria and the organisms with an oxidative, but not photosynthetic, metabolism are less pronounced than those between *Thio-* and *Athiorhodaceae*. Yet, if one compares the metabolism of the purple bacteria in the dark with that of non-photosynthetic organisms, it is impossible to point to even one small but characteristic difference.

Here one can, of course, argue that the division between purple bacteria and the *Eubacteriales* along physiological lines is simple and sharp, because it can be based upon the ability of the purple bacteria to metabolize by means of a photosynthetic process. This, moreover, fits the ecological facts; enrichment cultures of purple bacteria have never been achieved except under conditions of proper illumination, so that it is reasonable to accept the proposition that the photosynthetic mode of life represents the most important physiological characteristic of the group. The experiments discussed at the end of section *a* (p. 48) bear this out convincingly.

But this, in turn, raises another problem, to which Czurda and Maresch (20) have called attention. The normal requirements for growth of the *Athiorhodaceae* include the presence of organic substances. For a long time it has been customary to refer to such organisms as heterotrophs, in contrast to the autotrophic living beings whose nutritional needs can be satisfied entirely by inorganic compounds. Now it is obvious that an autotrophic bacterium must necessarily synthesize all its cell constituents exclusively from carbon dioxide and other minerals, whereas such a complete synthesis would be superfluous for organisms living in the presence of organic matter. But if it were demonstrated that carbon dioxide plays an important role also in the synthetic processes of organisms living in organic media it would be difficult to maintain the distinction.

In this connection Czurda and Maresch have reported an experiment from which they concluded that in a peptone medium the *Athiorhodaceae* utilize only carbon dioxide as a carbon source. This is a far-reaching conclusion, and certainly not justified by the experimental evidence. The experiment was planned so as to enable them to compare the development of seven strains of *Athiorhodaceae*, in the light and in the dark, under aerobic and anaerobic conditions, and in the presence and absence of carbon dioxide. The medium used was a 1% peptone "Vaillant" solution at pH 5.2 for the CO_2-free cultures; in a corresponding series the presence of carbon dioxide was insured by the addition of 0.25% $NaHCO_3$. In this series the pH of the medium was, however, raised to about 7.2, so that the conditions included an important extra variable apart from the presence or absence of CO_2. Two of the strains did not develop in any of the media used; the remaining five all grew in the medium with $NaHCO_3$, though only in the light; while only two yielded positive cultures in the illuminated but CO_2-free peptone solution. In table 7 the results are summarized.

I have already remarked that an acid medium either impedes or completely suppresses the development of the non-sulfur purple bacteria. It is, therefore, not surprising to find that the neutralized medium is superior to the acid one. The two strains which yielded positive cultures in the initially CO_2-free, acid solution developed considerably more slowly than in the bicarbonate-containing, neutral environment; also, the pH in the former had risen to 7.9 and 8.1. This is understandable since in the breakdown of the peptone ammonia is produced. The results indicate that these two strains were less sensitive to acid than

the others, or that the inoculum was large enough so that the organisms introduced could gradually create a more satisfactory environment as the result of their metabolism, or that a combination of these factors was operative. The delayed development is also logically accounted for by these possibilities. The failure of two strains—among which was one of *Rhodospirillum rubrum*—to grow under any conditions is not explained; it indicates that the experiment cannot be considered as having been conducted under satisfactory conditions. The same holds good for the negative results of all the cultures kept in the dark In the absence of comparable data on the development of the seven strains in neutral media in the absence of CO_2 it is hard to understand how the results obtained can be used to derive the conclusion that the three strains which developed in the medium neutralized with $NaHCO_3$, but not in the acid, CO_2-free solution, are obligatory carbon-autotrophs, and utilize only CO_2 and not the peptone as a carbon source.

It is not only the fact that the requisite controls are not represented in the experiment which makes this conclusion untenable. During the past 30 years it has been repeatedly shown that many microörganisms—indeed all that have been carefully tested—fail to grow in media which are kept rigorously CO_2-free. (See, for example, Gladstone *et al.* (88)). May one, then, deduce from such observations that all these organisms, molds, yeast, protozoa, and a large variety of bacteria, "are obligatory carbon autotrophs and utilize only carbon dioxide as a carbon source"? Certainly not; and yet this is what Czurda and Maresch did for the purple bacteria on the basis of experimental results which are in no way different!

TABLE 7

Résumé of Czurda and Maresch's results

	OXYGEN PRESENT			OXYGEN ABSENT		
	Light		Dark	Light		Dark
	CO_2-free	0.25% $NaHCO_3$	0.25% $NaHCO_3$	CO_2-free	0.25% $NaHCO_3$	
Number of positive cultures.....	2	5	0	0	5	0
Initial pH....................	5.2	7.2	7.2	5.2	7.2	5.2; 7.2

We must, consequently, conclude that Czurda and Maresch have not contributed satisfactory experimental evidence for their contention. Nevertheless, the theoretical problem raised by them is one which deserves attention. By Gaffron's experiments (1, 5) it was firmly established that illuminated suspensions of *Athiorhodaceae* utilize carbon dioxide during their metabolism of the higher fatty acids. It would thus be possible to infer that the organic substrates serve exclusively as hydrogen donors, and that the synthesis of cell material proceeds entirely from carbon dioxide through its photochemical reduction. But this is not necessarily the case. Gaffron's experiments do not exclude the possibility that "during the breakdown of these organic compounds there may be formed intermediate products which can serve immediately as raw material for some of the anabolic reactions" (52, p. 140–141). Foster, in 1940, expressed the same opinion: "It is far more likely that in the course of the oxidation of ... organic substrates there may be formed intermediate products which can be directly converted into cell materials. The recent studies on oxidative assimilation by colorless organisms furnish a very strong support for this view" (6, p. 134). Elsewhere (8) I have presented experimental evidence in favor of the concept that the organic substrate is, in fact, used by photosynthesizing *Athiorhodaceae*

in a manner analogous to, if not identical with, that in which it serves as a carbon source for non-photosynthetic organisms.

The fact remains, however, that in the light the non-sulfur purple bacteria can and do utilize carbon dioxide at least partly for the synthesis of cell materials. Furthermore, Foster's experiments have shown convincingly that special organic substances, such as secondary alcohols, are used exclusively as hydrogen donors, and not as building materials, since these substrates are quantitatively converted into the corresponding ketones which are left in the medium as metabolic end products. In addition to this we must consider the cases in which growth of *Athiorhodaceae* results from the oxidation of sulfide, thiosulfate, and hydrogen as incontrovertible evidence for a synthesis of cell substance from carbon dioxide as carbon source.

In view of the above considerations, it would thus appear possible to consider at least some members of the group as *potentially* autotrophic, if it were not for the fact that all the typical representatives require additional growth factors. The problem of proper terminology is hereby rendered much more difficult of solution. The strictest interpretation of the *designation "autotrophic" implies* an utter independence of other living organisms, *i.e.*, a completely mineral nutrition. In this sense the *Athiorhodaceae*, with the possible exception of a few types (see discussion in the preceding section), are not autotrophs. But the term has often—and not without justification—been used with reference to organisms capable of utilizing carbon dioxide for the synthesis of cell constituents. On this basis it is, however, most unsatisfactory to describe a bacterium which can grow in a strictly mineral medium by oxidizing molecular hydrogen as autotrophic, and a closely related organism as heterotrophic merely because for growth it requires the presence of, for example, 1 μg per liter of biotin. It will be conceded that the metabolism of the two organisms must be very similar indeed, and that it would, therefore, be undesirable to characterize their nutrition by terms which carry a connotation of profound differences. But if in this case an organism is admitted among the autotrophs because it requires only a very small amount of one single organic substance, and can produce all its other cell materials from CO_2 as the only carbon source, it becomes difficult to draw a sharp line of demarcation, because this would have to be settled on a quantitative basis. And just how much synthesis from CO_2 should be required to tip the balance in favor of one or the other term? The experience of the past five years has shown convincingly that all living organisms possess the ability to use carbon dioxide for some syntheses. As Werkman and Wood state it, "the close relationship between the heterotroph and the autotroph is becoming increasingly clear" (28, p. 12).

All this reflects the ever recurrent difficulty of carefully defining terms for use in scientific writing. Pirie's cogent remarks in a discussion of the terms "life" and "living" are worth quoting in this connection:

"Now, however, systems are being discovered and studied which are neither obviously living nor obviously dead, and it is necessary to define these words or else give up using them and coin others. When one is asked whether a filter-passing virus is living or dead the only sensible answer is: 'I don't know; we know a number of things it will do and a number

of things it won't and if some commission will define the word 'living' I will try to see how the virus fits into the definition.' This answer does not as a rule satisfy the questioner, who generally has strong but unfortunate opinions about what he means by the words living and dead" (89, p. 12).

In the present instance it seems to me preferable not to attempt a rigorous definition of the terms auto- and heterotrophic which may satisfy some, and upset others, but, after having called attention to the difficulties involved in the use of these words (see also 59), abstain from using them in order to characterize the metabolic properties of the *Athiorhodaceae*. What has so far been said concerning the general physiology of the group should, at any rate, give a far more complete picture of the behavior of the organisms than can ever be rendered by one single word.

6. THE PIGMENTS OF THE NON-SULFUR PURPLE AND BROWN BACTERIA

I. Introduction; physiological effects of the pigments

The characteristic colors of the non-sulfur purple and brown bacteria are chiefly due to the occurrence of two types of pigments. As far as has been ascertained, they appear to be distributed more or less evenly throughout the cytoplasm. By various methods it has been possible to extract and separate the main components. Since the pigment system is of fundamental importance for the physiological behavior of the organisms inasmuch as it is operative in the photosynthetic processes, a discussion of its properties appears pertinent.

Not until 1935 were definite chemical studies on the pigments reported. The earlier studies were primarily concerned with determinations of the optical properties of the bacteria themselves and of crude extracts, and with problems in terminology, although it is only fair to record that Engelmann's fundamental investigations on the relations between the physiological effects of light of different wavelengths and the absorption spectrum of the purple bacteria, dating from 1883–1888, laid the foundation for an interpretation of their physiology (90, 91).

It is a curious phenomenon that Molisch is so often credited with having supplied the first important contributions to our knowledge of these pigments. As Buder (9) has pointed out, all students of the purple bacteria, from Ray Lankester in 1873 on, have agreed on the complexity of the "bacteriopurpurin," the name used by Lankester to refer to the entire pigment system of the organisms. In a little-known publication Ewart (92), as early as 1897, clearly demonstrated that the purple bacteria contain both a green and a red pigment which can be separated by the use of proper solvents. Nadson (93) subsequently paid especial attention to the green, and his student Arcichovskij (94) to the red component, for which he proposed the name "bacterioerythrin," reserving the designation "bacteriopurpurin" for the complex in the sense of Lankester. Three years later Molisch (4), apparently rediscovering the presence of both green and red pigments in the purple bacteria, introduced the name bacteriochlorin for the green pigment, while limiting the term bacteriopurpurin to the red component. As a new contribution he produced evidence for the existence of two different red pigments, bacteriopurpurin α and β. In order to avoid confusion Buder (9) later proposed to standardize the terminology by accepting bacteriopurpurin to designate the complex pigment, bacteriochlorin the green, and bacterioerythrin the red components. At present these names have no more than an historical interest. The recent

investigations on the chemical nature have made it logical to coin terms more in keeping with the structure of the substances, about which more anon.

While the gradual development of methods for extracting and separating the green and red pigments led to their ultimate isolation as chemically pure compounds, and thus contributed greatly to the elucidation of their composition, a few reports were published which indicated that in the cells these components might be present in the form of a chemical combination which was destroyed by the treatment with various solvents. This attitude is particularly pronounced in the papers of Ljubimenko (21) and of Lévy, Teissier and Wurmser (95). Actually, the evidence presented by these workers is not convincing, although the latter investigators made it probable that the pigments occur as protein compounds, and that the protein component appeared to consist of globulin. The recent work of French (33, 45) has dispelled all doubt in this respect; it must now be accepted as firmly established that the organisms contain the pigments in the form of chromoproteins. It has not been definitively settled whether both red and green pigments are combined with the same or with different proteins.

That these pigments have an important physiological function was shown by Engelmann's observations on the behavior of purple bacteria in a spectrum (90, 91). Under special conditions the direction of their movements is governed by light; moving from a strongly illuminated section of the field into one more dimly lit, they rapidly reverse the direction of progression ("Schreckbewegung" in Engelmann's terminology). Since light of different wavelengths is absorbed to a different extent, and thus exerts the same influence as areas of different intensity, the result of exposing a suspension of purple bacteria to a spectrum is that after a while the organisms have accumulated in certain, rather narrow regions of the spectrum. From the behavior of the bacteria in and around these regions it is clear that the latter are experienced as "light," while the parts of the spectrum from which the cells gradually disappear, correspond to "dark" bands with respect to the sensitivity of the organisms. Engelmann proved that the accumulations occur in exact correspondence with the absorption spectrum of the bacteria. In view of the concept that only absorbed radiation can be physiologically active (law of Grotthus-Draper) this is, of course, not surprising. But special significance attaches to the fact that the aggregations were found to be most pronounced in the near infra-red region and least in the blue where, nonetheless, a strong absorption by the bacteria can be recognized. Concerning this last accumulation Engelmann wrote: "... und in günstigem Falle auch die Andeutung eines verwaschenen breiten Bandes zwischen etwa 0.55 und 0.52 (μ) kenntlich sind." (91, p. 162). The more extensive and careful experiments of Buder (9) have fully confirmed this behavior; the phototactic responses of the purple bacteria are most pronounced in the infra-red region though still perceptible in the blue.

This point is of interest because the separation of the green and red pigments has made it possible to ascribe the various absorption bands of the organisms to either one of the two types of pigments. Thus the work of Nadson, Molisch, Buder, and all later students has shown beyond a doubt that the green component is responsible for the infra-red absorption bands and for the one around 590 mμ, while the bands in the shorter wavelength region are exclusively due to absorption by the red pigments.

The occurrence of bacterial accumulations in the range below 590 mμ consequently suggests that the red pigments may be weakly functional in causing phototactic reactions, albeit the green component is far more active in this respect. Now, the close connection of the latter with the photosynthetic metabolism of the purple bacteria has been conclusively established by the measurements of French (32) of the rate of photosynthesis in light of different wavelengths from which it not only follows that the green pigment is photosynthetically functional, but also that light absorbed by the red components is completely lost for the photosynthetic process. The important study of Schrammeck (24) on the quantitative aspects of phototaxis by purple bacteria has demonstrated how extraordinarily sensitive the organisms are to differences in light intensity. But because his work was done with unfiltered light from electric bulbs it is as yet impossible to reach any more definite conclusions as to the relative effects of light absorbed by the green and the red pigments on phototaxis. It is not hard to believe in the existence of a carotenoid-sensitized photobiological response, especially in view of the intimate relation of phototropic responses of green plants with carotenoid pigments as established in many instances. The anomalous situation in the case under discussion is that here two fundamentally different pigments, one of which fulfills a specific function in metabolism which the other is known not to possess, should cause qualitatively the same physiological effect, though different in intensity. French (32) has hinted at a possible explanation on the basis of the assumption that the phototactic reaction might be complicated by secondary, chemotactic effects. Similar complications could also arise from superimposed thermotactic phenomena. Much careful work will, however, be necessary before this problem can be more satisfactorily interpreted.

If there be some doubt concerning the existence of a direct phototaxis of the purple bacteria caused by the red pigments, no hesitation is justified in accepting the view that for photosynthesis these organisms can use only light absorbed by the green pigment. First and foremost this statement is supported by French's exact studies (see above). Additional evidence has been furnished by experiments on growth of various purple bacteria in a spectrum. (Eymers and Wassink, mentioned in (96); many unpublished experiments of Dr. W. Arnold). The growth regions coincide accurately with the absorption bands of the organisms except that at wavelengths below 570 mμ growth does not occur.

For a more detailed treatment of the photosynthetic reaction of the purple bacteria the reader should consult other sources (8, 97, 98).

II. The green pigments

When Ewart had found (92) that a green pigment could be extracted from purple bacteria by alcohol, he carried out some further simple experiments with the solution. The pigment was easily transferable to ether or benzene; the solutions showed a red fluorescence, faded rapidly when exposed to light in the presence of oxygen, and turned brown on treatment with acid or alkali. From these observations he concluded cautiously that "a green dye apparently identical with chlorophyll" was present in the organisms.

The green pigment which Nadson (13) later studied was, however, distinctly different from chlorophyll with regard to its absorption spectrum, which showed a pronounced band around the Fraunhofer D-line (600–580 mμ). Molisch arrived at the conclusion that the green component could not be chlorophyll, at the same time admitting that some characteristics of the extracted substance were strongly reminiscent of the typical plant pigment: "Die schöne grüne Farbe, die Ausschüttelungsversuche und die schwache, rote Fluoreszenz könnten auf die Vermutung führen, dass man es beim Bacteriochlorin eigentlich mit Chlorophyll zu tun habe. Die spektroskopische Prüfung und manches andere spricht aber ganz dagegen . . ." (4, p. 79). Apart from the absorption spectrum of the green solutions, which is totally different from that of chlorophyll, a clear distinction was indicated by the treatment of the solutions with strong alkali. Molisch had observed that chlorophyll solutions at first turn brown, and later become green again when subjected to the action of alkali. (Molisch's chlorophyll test, later known as the "phase test"). The green extracts from purple bacteria responded to the addition of alkali by turning brown, but never resumed a green color. The same difference was exhibited by the effect of acid. While recognizing the profound differences in the behavior of solutions of "bacteriochlorin" and of chlorophyll, he nevertheless stated: "womit aber nicht gesagt sein soll, dass zwischen Bacteriochlorin und Chlorophyll nicht auch verwandschaftliche Beziehungen bestehen könnten" (4, p. 80).

One finds the same reasoning in Buder's discussion of the green bacterial pigment. He, however, recognized another important problem which Nadson and Molisch had not raised. Much more aware of the importance of Engelmann's phototaxis experiments, which Buder repeated and expanded, the latter realized that it was still uncertain whether the green or the red component of the purple bacteria pigment system was responsible for the marked and spectacular absorption in the infra-red region. Direct measurements of the infra-red absorption by the green solutions were not carried out, however. Buder tried to solve the problem by studying the phototactic accumulation of the purple bacteria in light filtered through solutions of the green and red components respectively, expecting that the solution of the particular pigment which was characterized by the infra-red absorption bands would prevent the organisms from exhibiting the corresponding phototactic bands. Much to his surprise he found that the accumulations in the infra-red region occurred behind both the solution of bacteriochlorin and that of bacterioerythrin, but were eliminated if a filter of living bacteria or a ferrous sulfate solution was used. As an interesting possibility Buder points out that perhaps a third pigment is present in purple bacteria which absorbs mainly in the infra-red:

"Mit Sicherheit lässt sich aus diesem unerwarteten Ausfall der Versuche nicht allzuviel schliessen. Er legt aber eine Möglichkeit, die man bisher zu berücksichtigen überhaupt keine Ursache hatte, als Gegenstand weiterer Prüfung nahe. Es wäre nämlich zu untersuchen, ob etwa eine dritte Komponente des Bakteriopurpurinkomplexes vorhanden ist, die sich durch starke Absorption der fraglichen Gebiete des Infrarots auszeichnet, in Alkohol und Schwefelkohlenstoff aber nicht oder nur sehr schwer löslich ist. Da das

sichtbare Absorptionsspektrum des Bakteriopurpurins sich durch die Übereinanderlegung der Spektra des Bakterioerythrins und -chlorins, wie es scheint, ziemlich restlos erklären lässt, bliebe für ein drittes "Pigment" keine wesentliche Absorption im sichbaren Bereiche mehr übrig. Es müsste also ein nahezu farbloser Körper sein" (9 p. 549).

It may here be remarked that later studies have shown that actually the green pigment is the one which is responsible for all infra-red absorption bands, but that its absorption characteristics undergo considerable changes upon extraction with alcohol.   In this connection special attention should be given to the extensive and careful studies of Wassink et al. (96, 99, 100).   They demonstrated that the green alcoholic extracts of a variety of pure cultures of purple bacteria exhibit only one absorption maximum in the infra-red, situated at 774 mμ.   The living cells, on the other hand, show two or more distinct maxima in this region, and their positions vary with the species.   The differences may be considerable, as shown by the data summarized in table 8.

TABLE 8

Infra-red absorption bands of various species of purple bacteria (after Wassink et al.)

STRAIN	ABSORPTION MAXIMA, mμ						
Thiorhodaceae							
Type 1	895			855–850		796	
Type 2	895		865		804		
Athiorhodaceae							
Type 1		892–885		850		799	
Type 2			880–863		802		
Type 3			875			800	
Alcoholic extract of all types							774

Aqueous extracts of the purple bacteria pigments, prepared by supersonic disintegration or by grinding of the cells (French 33, 45, 101; Katz and Wassink (99, 100)), display absorption spectra which are in excellent agreement with those of the intact cells from which they are obtained.   Since the 774 mμ absorption maximum of the alcoholic extracts is not found by spectroscopic examination of any of the living purple bacteria, it is reasonable to believe that the alcoholic solutions contain a "decomposition product" of the pigment complex.

This is essentially the same conclusion at which Ljubimenko (21) had arrived in 1921.   His studies were undertaken in the hope of establishing a relationship between the pigments of the purple bacteria on the one hand and the green plant chlorophylls and the green bacteria pigment on the other hand.   The results obtained led him to express his views as follows: "Il est probable que le bactério-pourpourine, matière colorante des bactéries pourpres, est intimement liée aux substances albuminoides du protoplasma.   Sous l'action de divers dissolvants qui rompent cette liaison par une simple coagulation de ces substances, la bactériopourpourine se décompose en une série de dérivés de couleur bleu et rouges" (21, p. 119).

A closely similar situation exists with respect to the absorption spectra of chlorophylls a and b in green plants and in alcoholic solutions. In the 1920's Ljubimenko derived from the existing discrepancies the conclusion that chlorophyll occurs in the plant in the form of a protein complex. The later careful studies of Mestre (105–107) have contributed important experimental support for this concept. Furthermore, the investigations of a number of enzymes by Warburg and his school have demonstrated the occurrence of a pronounced shift towards longer wavelengths in the position of the absorption maxima of the prosthetic groups following their recombination with the protein carrier. All this tends to lend conviction to the idea that the disappearance of the characteristic infra-red absorption maxima of intact purple bacteria or their aqueous extracts upon extraction with alcohol, coincident with the appearance of a new band in the green alcoholic solution, is a result of the severance of the pigment from a protein-dye complex.

These investigations on the absorption spectra of living organisms, aqueous extracts, and alcoholic solutions furnish a complete explanation of the results which Buder obtained in his studies on the "accumulation bands" of purple bacteria in the infra-red region behind an alcoholic bacteriochlorin solution. The latter is, namely, transparent to wavelengths which are absorbed by the living cells, so that the typical phototactic aggregations at wavelengths longer than 780 mμ can still occur in light that has passed through the alcoholic extract. And, although it is practically certain that the infra-red absorption in the organisms is principally due to the green component, one might still admit that Buder's idea of the existence in the cells of a third component, responsible for the absorption maxima in the infra-red only, was essentially correct. With the possibility of identifying this "third component" with proteinaceous constituents, and of envisaging the pigment system of the organisms as a protein-pigment complex, the experimental evidence of various investigators, and the concepts of Buder, Ljubimenko, and others have thus been harmoniously correlated.

While in most of the foregoing work similarities as well as differences between the green pigment of the purple bacteria and chlorophyll had been stressed, an approach to the chemical nature of the "bacteriochlorin" was not made until 1934.

It is true that in 1925 Lévy *et al.* (95) had carried out some studies on the green component, obtained by extraction of purple bacteria with methanol, but the tests were crude, and the results difficult to interpret. They concluded: "D'après ses propriétés chimiques, il nous semble que la bactériochlorine doit probablement être considérée comme un pigment parent des carotinoides, si l'on veut comme un 'lipochrome' qui ne serait pas un carotinoide proprement dit" (95, p. 304). But it remained for Schneider to establish the close similarity of the green pigment with the plant chlorophylls on the basis of chemical analyses of the purified product (108, 109), at the same time proposing a change of name to bacteriochlorophyll: "Die Untersuchung hat ergeben, dass der grüne Farbstoff der Purpurbakterien dem Chlorophyll ausserordentlich nahe verwandt ist. Er ist demnach richtiger als 'Bakteriochlorophyll' zu bezeichnen, da der Name Chlorin schon für andere Körper der Chlorophyllreihe vergeben ist" (109, p. 222).

The most important results of Schneider's investigation are the demonstration that the green pigment is a pyrrol dye with porphin nucleus, containing mag-

nesium in complex combination. The combustion analysis led to the formula $C_{55}H_{72}O_6N_4Mg \cdot 1H_2O$. From the presence of 6 oxygen atoms Schneider inferred that bacteriochlorophyll is more closely related to the green plant chlorophyll b than to chlorophyll a. By preparing a large number of conversion products along lines which had previously been worked out (especially by Willstätter and Stoll, Stoll and Wiedemann, and H. Fischer et al., during their studies on chlorophyll), Schneider substantially supported the contention that the green component of the purple bacteria pigment is a chlorophyll. For example, treatment of bacteriochlorophyll with dilute acid results in the loss of the magnesium atom from the molecule with the formation of bacteriopheophytin; the latter, still a wax, can be converted into the readily crystallizable bacteriopheophorbid, and this, in turn, into its methyl ester; while from these porphin compounds various porphyrins can be obtained by the usual methods.

At about the same time H. Fischer and co-workers commenced the publication of their investigations on the chemistry of bacteriochlorophyll, through which not only important details were added, but also misconceptions were cleared up, and ultimately the exact chemical structure of the molecule became fairly well established (110–116). Thus they demonstrated the presence of a phytyl group which can be split off by "chlorophyllase," and succeeded in carrying out the degradation of bacteriochlorophyll to oxo-pheoporphyrin a$_5$, the latter a typical derivative of the chlorophyll a series. The last-mentioned conversion can be achieved without having recourse to the "oxo-reaction," whereby the vinyl group of chlorophyll a is converted into an acetyl group. This transformation of bacteriochlorophyll into a substance identical with one obtained from chlorophyll a established that the bacterial pigment should be regarded as related to chlorophyll a rather than to b. The sixth oxygen atom in the bacteriochlorophyll is not present in a formyl group, as it is in chlorophyll b, but in an acetyl group which occupies the same position in the molecule as does the vinyl group in the green plant chlorophylls. Also, it was found that bacteriochlorophyll contains two hydrogen atoms in excess of chlorophyll a which can be very easily removed. The various compounds of the bacteriochlorophyll series then give rise to dehydro-derivatives, still more closely related to similar members of the chlorophyll a series. How close this relationship is was shown by the recent syntheses of dehydro-bacteriopheophorbid and dehydro-bacteriochlorin from derivatives of chlorophyll a, and by the beautiful experiments in which the acetyl group of dehydro-bacteriopheophorbid was transformed into a vinyl group, thus practically achieving its conversion into chlorophyll a.

In connection with the successful interconversion of the acetyl compound via the α-hydroxy stage to the vinyl derivative, according to the reaction:


,
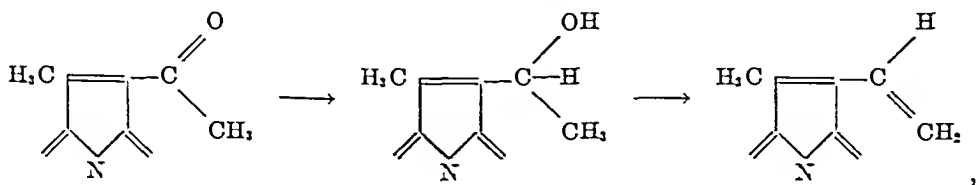
Fischer, Mittenzwei, and Hevér wrote:

"Die α-Oxy-körper bilden somit neben den Acetylderivaten eine neue Brücke zwischen Chlorophyll a and Bacterio-chlorophyll. Durch Wasserabspaltung der 'analytischen' α-Oxyverbindungen im Hochvakuum zu Chlorin e₆-triester konnte eine weiterewichtige Zwischenstufe in der nun folgenden Übersicht der bestehenden Übergänge dargestellt werden:

Phäophorbid a ⇌ Chlorin e₆ ⇌ 2,α-Oxychlorin e₆ ⇌

⇌ 2-Acetyl-chlorin e₆ ⇌ Dehydro-bacterio-phäophorhid a.

Da Phäophorbid a bereits von uns reversibel in Chlorophyllid a übergeführt ist, und von Phäophorbid aus auch in Phäophytin, bzw. Chlorophyll a, ist damit der endgültige Beweis geliefert für die vollständige Übereinstimmung der Konstitution von Dehydro-bacteriophäophorbid a und Chlorophyll a. Noch steht der definitive Beweis aus dass bei der Dehydrierung von Bacterio-chlorophyll keine Konstitutionsänderung eintritt. Angesichts der grossen Labilität von Bacterio-chlorophyll halten wir dies nicht für gänzlich ausgeschlossen" (116, p. 162).

The most probable formula for the dehydrobacteriopheophorbid, according to Fischer et al., thus is:
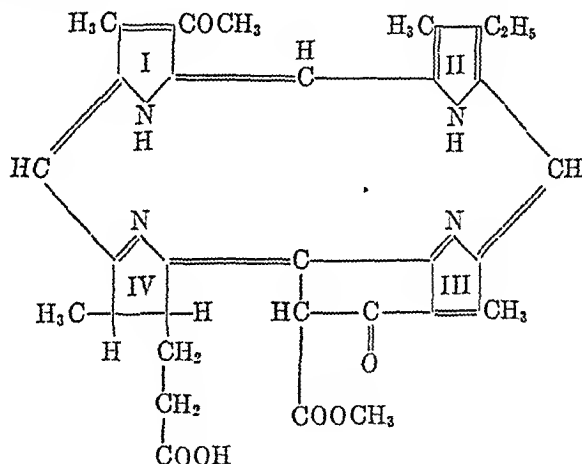
With a phytyl group substituting for the acidic hydrogen of the propionic acid attached to pyrrol ring IV, and the two hydrogens in rings I and II replaced by Mg, this structure should also closely represent the formula of the bacterio-chlorophyll itself, with the position of the two extra hydrogen atoms still in doubt.

Relevant to these views are also the spectroscopic investigations of various derivatives of chlorophyll a and bacteriochlorophyll by Stern and Pruckner (162). Although so far restricted to measurements in the visible region, the position of the absorption bands supports the chemical evidence for the occurrence of an acetyl group in bacteriochlorophyll, located in the same place where chlorophyll a carries a vinyl group.

Schneider, considering bacteriochlorophyll as more closely related to chloro-phyll b than to a, looked for a second green component in the purple bacteria, and presented some evidence for its existence. According to the studies of

Fischer *et al.* this substance would, however, be a decomposition product of genuine bacteriochlorophyll and not a regular constituent of the pigment system. The latter investigators could find no indications for the occurrence of more than one bacteriochlorophyll in *Thiocystis violacea*, one of *Thiorhodaceae*. Since Schneider had investigated the pigment of *Athiorhodaceae* cultures, which undoubtedly represented a mixture of different species, it was entirely possible that the conflicting evidence might have been due to the fact that the various members of the groups of *Thio-* and *Athiorhodaceae* do not all contain identically the same bacteriochlorophyll. The marked variations in the infra-red absorption spectrum of different organisms, commented upon before, could readily be considered as experimental support for this assumption.

Nevertheless, the evidence obtained to date does not bear this out. In the first place, chromatographic analyses of bacteriochlorophyll extracts from pure cultures of several species of non-sulfur purple bacteria have made it appear most unlikely that any one species contains more than one bacteriochlorophyll. Furthermore, the absorption spectra of the alcoholic solutions are, as far as has been ascertained, identical. This also includes the alcohol extracts from members of the *Thiorhodaceae* (See also (99)). Thirdly, the various bacteriochlorophyll extracts have been used for the preparation of pure bacteriopheophytin, bacteriopheophorbid, and bacteriomethylpheophorbid. A careful comparison of the absorption spectra, melting points, mixed melting points, and analytical data of these products again has yielded no indication for the existence of different bacteriochlorophylls.[4] Although it is true that until now such comparative studies have not been carried out with pure bacteriochlorophyll preparations obtained from different species, the present information thus suggests that the various purple bacteria contain the same bacteriochlorophyll. (Many unpublished results, in part with Drs. E. Wiedemann and W. A. Arnold, and referred to in (104) and (117).) In view of the fact that the combination of the pigment with protein radically alters the absorption characteristics, it seems most reasonable to assume for the present that the differences in infra-red absorption exhibited by various species must be ascribed to compounds of one bacteriochlorophyll with different proteins, a conclusion with which French (33) and Wassink *et al.* (100) concur.

III. The red and yellow pigments

If a mass of purple bacteria is treated with concentrated sulfuric acid, the material soon turns blue. This reaction, first reported by Winogradsky (11), became the basis for the belief that the purple bacteria pigment would be a lipochrome. Nadson, while admitting the validity of the observations, nevertheless pointed out that the inference was at best premature: "From this it may be deduced that there are, indeed, lipochromes in the cells of these bacteria. But one should not conclude that bacteriopurpurine is itself a lipochrome"

[4] This statement is correct only for the green pigment of the purple bacteria. The green sulfur bacteria contain a chlorophyll which is most certainly not identical with either bacteriochlorophyll or chlorophylls a and b.

(93, p. 112).   Both the solubility and absorption spectrum characteristics argued strongly against it.   This state of affairs led to Arcichovskij's study (94) of the lipochrome constituent, "bacterio-erythrin," which he obtained in crystalline form and characterized so well spectroscopically that even to-day it is possible to identify this pigment with one of the various carotenoids now known to occur in purple bacteria.   This is of importance because it has become clear that, contrary to what appears to be true for the green component of their pigment system, the purple bacteria contain a variety of carotenoids.   Molisch (4) distinguished two, but to-day it is certain that many more exist.

Again, the early studies on the red pigment components dealt with their properties chiefly as far as their behavior towards various solvents and their absorption spectra were concerned.   More recently investigations have been carried out with a view to establishing the chemical nature of the substances. The first of these (62, 118) led to the isolation in a crystalline form of a carotenoid pigment from *Rhodospirillum rubrum*.   On account of its origin and chemical properties the compound was named spirilloxanthin; it is a dark purple, beautifully crystalline substance, easily decomposed by light and oxygen, and its absorption spectrum agrees with that of Arcichovskij's bacterio-erythrin, and with Molisch's "bacteriopurpurine α."

Soon afterwards Karrer and Solmssen (119, 120) obtained a similar substance, which they named rhodoviolascin, from mass cultures of *Rhodovibrio*.   The melting points, solubility characteristics, and absorption spectra of spirilloxanthin and rhodoviolascin show a striking resemblance.   The analytical data, especially the results of carbon and hydrogen determinations, are not in such good agreement, however.   This is the reason why different formulas have been proposed for spirilloxanthin (62) and rhodoviolascin (119–123).   According to the analyses of van Niel and Smith the former compound can best be represented by $C_{48}H_{66}O_3$ with 15 double bonds per molecule, while Karrer and Solmssen's results suggest $C_{42}H_{60}O_2$, containing 13 double bonds, for rhodoviolascin.   It should be pointed out that the presence of two methoxyl groups in the latter substance (120) has been corroborated also for spirilloxanthin (van Niel and Wiedemann, unpublished).   The number of double bonds is computed from hydrogenation data, and the result naturally depends upon the assumed molecular weight of the substance.   Thus, the values of van Niel and Smith are reduced to 12.8 and 12.9 if the molecular weight of spirilloxanthin were 596 instead of 690.5, while Karrer and Solmssen's data, upon recalculation for a substance with the higher molecular weight, would indicate the presence of 15 double bonds per molecule.

There are a few observations which, for the time being, favor a skeptical attitude to the most recent formulation of the structure of rhodoviolascin by Karrer and Koenig (123).   One is that the position of the absorption bands would be in better agreement with a larger number of double bonds (see also (62)).   Karrer and Solmssen remark in this connection: "… man wird bis auf weiteres die Annahme machen müssen, dass die langwelligere Lichtabsorption durch die Methoxylgruppen mitbestimmt ist" (120, p. 4).   A second difficulty lies in the fact that spirilloxanthin appears to contain one active hydrogen atom per

molecule (van Niel and Smith (62); van Niel and Wiedemann, unpubl.) which could be readily understood if the molecule contained one hydroxyl group, but for which there is no place in Karrer's formulation. Thirdly, the oxidation of rhodoviolascin by ozone yields acetone (120), which is contrary to the expectation if the formula proposed (123) were correct. It is true that the analysis of the products resulting from the oxidation of rhodoviolascin by permanganate (122, 123) makes it highly probable that the pigment contains a bixin structure, but this does not necessitate a structure of the rest of the molecule analogous to that of the better known carotenoids.

It is, of course, possible that spirilloxanthin and rhodoviolascin are not identical. In 1936 I presented Prof. Karrer with about 500 mg of pure spirilloxanthin, and asked him to compare this substance with rhodoviolascin. The determination of a mixed melting point would have gone far to settle this problem, but Karrer has not yet reported the results.

So much for the best known of the red pigments. In the extracts of *Rhodospirillum rubrum* five additional yellow and red pigments have been found (62), but until now no one has been obtained in sufficient quantity to allow its closer study. From spectroscopic observations one seems to bear a resemblance to spirilloxanthin, with all three absorption maxima shifted toward shorter wavelengths.

Karrer and Solmssen have obtained several other red pigments from *Rhodovibrio* mass cultures. The main distinctions are still based on the position of absorption maxima, and it seems premature to consider them all as chemically distinct individuals. This is the more true because the melting points of some of the fractions, as well as the position of the major absorption bands are very similar. Only one, rhodopin, has been obtained in a pure enough state to make the analyses significant; they indicate an empirical formula $C_{40}H_{58}O$ or $C_{40}H_{56}O$. Originally (119) Karrer and Solmssen claimed one methoxyl group per molecule of this pigment; in a later publication (121) the "small" methoxyl content of the original sample is ascribed to the admixture of rhodoviolascin. Since a methoxyl-free rhodopin would require approximately a half-and-half mixture with rhodoviolascin in order to account for the earlier found methoxyl content, the explanation seems extremely far-fetched. In addition, van Niel and Wiedemann (unpubl.) have isolated a red pigment with the characteristics of rhodopin from certain cultures of *Athiorhodaceae* which do not produce spirilloxanthin (rhodoviolascin); and in spite of the absence of the di-methoxy compound the beautifully crystalline substance appears to contain one methoxyl group.

The studies of Schneider (124) on the carotenoids of *Athiorhodaceae* may also be mentioned as providing further evidence for the complexity of the pigment system. Isolation of individual components was not attempted; the chromatographic analysis of extracts showed, however, the presence of a mixture of various colored substances.

Summarizing the present status of our knowledge of the red components of the purple bacteria pigments it may be said that the information is as yet conflicting and indefinite. None of the formulas so far proposed has been suffi-

ciently well substantiated, and it would be uncritical to go beyond the assertion that several different individual components have been met with. For the sake of convenience I have collected the information on absorption spectra in table 9.

Perhaps the most significant general conclusion that can be drawn from an inspection of table 9 is that by a spectroscopic examination three groups of red pigments can be distinguished. The first, corresponding to Arcichovskij's bacteriocrythrin and Molisch's bacteriopurpurin α, appears to be represented by spirilloxanthin and rhodoviolascin. The second comprises the pigments of the bacteriopurpurin β type. Among these Karrer's rhodopin probably constitutes the quantitatively predominant type; but the group, spectroscopically characterized by the position of its long-wavelength absorption band at 545–550 mμ (in CS$_2$), is likely to contain a number of chemically distinct pigments. The third group is composed of orange and yellow pigments such as reported by

TABLE 9

Absorption spectra of the "red" constituents of the pigments from purple bacteria

PIGMENT	MELTING POINT	WAVELENGTHS AT ABSORPTION MAXIMA IN VARIOUS SOLVENTS														REFER.
		CS$_2$			CHCl$_3$			C$_6$H$_6$			EtOH			Petroleum ether			
Bacterio-erythrin		570	530	490							527	493					94
Bacteriopurpurin α .		565	530		540	510											4
Spirilloxanthin	219	569	531	501	540	506	479	545	508	479	529	494		526	493	465	62
Rhodoviolascin ..	218	573	534	496	544	507	476	548	511	482	526	491	(405)				119
Bacteriopurpurin β		545	500		520	490											4
Bacteriocrythrin β		545	505	485													9
Rhodopurpurin	161–162	550	511	479	523	487		527	490					502	472		119
Rhodopin	171	547	508	478	521	486	453				505	474		501	?	440	122
Rhodovibrin .	168	556	517														121
2nd pigment from *Rhodospirillum rubrum*		551	518	485													62
Flavorhodin	111–113	502	472	441	482	453					472	443		470	442		122

van Niel and Smith (62) and by Schneider (124). Flavorhodin (122) is the only constituent which has so far been obtained in crystalline form. This must be ascribed to the fact that in the pigment extracts so far studied the red and purple components were predominant.

It is, however, to be expected that a continuation of the study of the carotenoids produced by different species of *Athiorhodaceae* will reveal cases in which the orange and yellow pigments are produced in far greater quantity than the red ones. In general, it seems likely that various strains differ markedly in the composition of their pigment system as far as the red components are concerned. This is substantiated by a number of observations. Firstly, Molisch obtained extracts from pure cultures of *Rhodobacillus palustris* which must have contained chiefly spirilloxanthin, and *not more* than small amounts of other carotenoids because the absorption spectra reveal only the characteristic bacteriopurpurin α bands. Similarly, Molisch's discovery of bacteriopurpurin β was possible by

extracting pure cultures of a *Rhodospirillum* species which obviously contained little or no spirilloxanthin.   Furthermore, the study of the red pigments of *Rhodospirillum rubrum* (62) showed convincingly the vast preponderance of spirilloxanthin among its carotenoids.

Such observations fit in well with the results of an examination of the cultures of different types of non-sulfur purple bacteria.   In the section on the morphological characteristics of this group, it has already been remarked that they can in part be distinguished by the color of the growth.   Especially the careful spectroscopic studies of French (33, 45, 101, 125) on pure cultures of a number of species have shown that three types can be differentiated by the position of the long-wavelength absorption bands of the carotenoids, respectively at 550, 530, and 500–510 mμ.   I have spectroscopically examined thousands of cultures, though much more cursorily, and believe that French's measurements can be used to characterize three distinctive groups of *Athiorhodaceae*.

While these various observations are strongly indicative of significant differences in the composition of the pigments of various *Athiorhodaceae* species, they do not rule out the possibility that in addition to one strongly predominant type of pigment a number of other carotenoids would be formed as well by many or all strains, though in considerably smaller amounts.   From a perusal of the publications of Karrer and Solmssen it would, however, appear as if both rhodoviolascin and rhodopin were produced in more or less equal quantity by some representatives of the purple bacteria, and that the composition of the pigment complex of any one species is extremely variable.   The following quotations bear this out.

"Auch die Zusammensetzung der den Rhodovibriokulturen entzogenen Carotinoidmischungen ist bei verschiedenen Ansätzen in qualitativer und quantitativer Hinsicht Schwankungen unterworfen; so konnte, worauf wir früher schon verwiesen, Flavorhodin öfters nicht festgestellt werden.   Gelegentlich beobachteten wir eine Carotinoidfraktion, die mit β-Carotin identisch zu sein scheint, in anderen Ansätzen aber nicht gefunden wurde" (121, p. 1019).

"Seither wurde ein Thiocystisstamm in grösserem Masstab bei uns weitergezüchtet und dessen Carotinoidgemisch untersucht.   Dieses erwies sich als ebenso kompliziert zusammengesetzt wie jenes der Rhodovibriobakterien und schien letzterem auch in der Zusammensetzung nahezukommen" (121, p. 1020).

"Mengenmässig am stärksten vertreten sind Rhodoviolascin und Rhodopin, die somit in erster Linie für die Färbung der Bakterien verantwortlich sind.   Die Ausbeuten betragen für diese beiden gereinigten Pigmente je 20–30 mg pro 300 Liter reifer Nährlösung" (121, p. 1019).

Nevertheless, these remarks are decidedly misleading.   When Karrer and Solmssen base their observations on results obtained with mass cultures of *Rhodovibrio*, it must be kept in mind that their cultures did not in any way represent pure cultures of a *Rhodovibrio* species.   Undoubtedly the major part of the organisms that developed in these mass cultures consisted of purple bacteria.   But small and uncontrolled variations in the culture conditions must have had a pronounced effect on the specific composition of the bacterial flora.   In fact, some statements in their papers make it obvious that *Thio-* as well as

Athiorhodaceae were present in these *"Rhodovibrio"* cultures. Hence their investigations cannot be interpreted in terms of the composition of the pigment complex of any one species of non-sulfur purple bacteria. The same holds true for their study of the red pigments of the sulfur purple bacterium *Thiocystis*. Here again, the mass cultures must have contained various representatives of *Thio-* and *Athiorhodaceae*. One should, therefore, expect that they found a mixture of carotenoids, produced by various species, and in unpredictable proportions.

It is significant that in a later paper they report: "Herr Peter R. O. Bally hatte die Freundlichkeit, uns "roten" Schlamm vom Nakura-See in Kenya Colony zu senden. Dieser lieferte mit Chloroform oder Schwefelkohlenstoff einen roten Extrakt, dessen scharfes Absorptionsspektrum mit demjenigen des Rhodoviolascins genau übereinstimmte. Andere Carotinoidbanden liessen sich nicht nachweisen. Es wird zu prüfen sein, ob hier andere Purpurbakterien vorliegen oder ob die Umweltsbedingunen die ausschliessliche Bildung von Rhodoviolascin begünstigt haben" (123, p. 462–463). From the fact that the material investigated consisted of a natural mass culture of sulfur purple bacteria it is safe to infer that it consisted mostly of *Thiorhodaceae*, not contaminated to a quantitatively appreciable extent with non-sulfur purple and brown bacteria. Consequently, the extracts also did not show the variety of pigments represented in the above-mentioned mixed cultures.

It is obviously futile to attempt a satisfactory description of the pigments of different *Athiorhodaceae* species until the individual carotenoids have been adequately characterized. And it appears to me that the most expedient approach to this problem will be a study of the pigments produced by different species when grown in pure culture.

So far, the red pigments have chiefly been characterized by the absorption spectra of their solutions in organic solvents. Yet this is not the form in which these substances occur in the bacterial cells. That the absorption bands of living bacteria can be correlated with the occurrence of specific pigments follows from the observations of Molisch, Buder, and French. However, here again one meets with a certain complexity which is necessary to indicate.

In connection with the discussion of the absorption spectra of bacteriopurpurin α and β, Molisch stated: "Bringt man die Spektren des Bakteriochlorins und des Bakteriopurpurins (in Schwefelkohlenstoff) zur Deckung, so resultiert dann so ziemlich des Spektrum der lebenden Bakterien.... Untersucht man anstatt des Rhodospirillum eine Aufschwemmung des Rhodobacillus palustris in Reinkultur, der, die wir wissen, nicht wie das rote Spirillum Bakteriopurpurin β, sondern die Modifikation α enthält, so erscheint das Spektrum dem des Rhodospirillums im wesentlichen gleich, man findet nur die Absorptionsbänder des Bakteriopurpurins, entsprechend der hier vorkommenden Modifikation α etwas gegen Rot verschoben" (4, p. 83–84).

Now, the only absorption spectrum of a bacterial suspension which is reproduced in Molisch's treatise is one of a *Rhodospirillum* species; no data are presented with respect to the position of the bands of a *Rhodobacillus palustris* suspension beyond the rather vague reference to a "slight shift towards longer wavelengths." The longest wavelength absorption band due to the red pigments of Molisch's *Rhodospirillum* culture is clearly around 545–550 mμ, which is in good agreement with French's determinations both for the intact bacteria

and for the proteinaceous, aqueous extracts obtained by grinding cells of *Rhodospirillum rubrum* (550 mμ). But here the first inconsistency appears. For Molisch's *Rhodospirillum* is alleged to contain bacteriopurpurin β, while the *Rhodospirillum rubrum* culture used by French is the identical strain which had previously (62) been shown to contain principally spirilloxanthin, and the latter is spectroscopically identical with Molisch's bacteriopurpurin α! It is thus easy to understand why Molisch contended that the carotenoid absorption bands in carbon bisulfide should be used in order to obtain the agreement between the spectral characteristics of the bacteria themselves and of the superimposed green and red extracts, because the long wavelength absorption maximum for bacteriopurpurin β in carbon bisulfide is situated at 545 mμ. But such a procedure fails to account for French's measurements; his *Rhodospirillum rubrum* strain should accordingly exhibit an absorption band around 570 mμ, since this is the position of the corresponding maximum of spirilloxanthin (bacteriopurpurin α) in carbon bisulfide. The absorption spectrum of *Rhodospirillum rubrum* would, therefore, show a far better correspondence with that of the combined extracts if the carotenoid fraction were dissolved in benzene (maximum for spirilloxanthin 545–550 mμ) instead of in carbon bisulfide.

Furthermore, a band characteristic for spirilloxanthin in carbon bisulfide (570 mμ) has never been encountered in the examination of living cultures or of aqueous cell extracts of any of the non-sulfur purple bacteria. There is also disagreement in the data on the absorption bands of *Rhodobacillus palustris*. Spectroscopic examination of several of my strains, closely resembling this species, reveals an absorption maximum at 530 mμ rather than at 550 mμ, just as do the aqueous extracts of *Rhodovibro* cultures, reported by French (33) who used a strain of *Rhodovibrio* which has, in my experience, proved almost indistinguishable from *Rhodobacillus palustris*. Extracts of the carotenoid pigments of these representatives appear to contain rhodopin (bacteriopurpurin β) rather than spirilloxanthin. Here again a reasonable agreement in the position of the maximum is obtained with benzene solutions of the carotenoids; the long wavelength absorption maximum of rhodopin in this solvent is situated at about 530 mμ.

Had Molisch inadvertently interchanged the legends for the designation of the absorption spectra of the red pigments extracted from *Rhodospirillum* species and *Rhodobacillus* respectively these discrepancies would automatically have resulted. It cannot now be decided whether this may have occurred. However, in that case another and independent observation by Buder presents new difficulties. In his studies on the accumulation of purple bacteria in a spectrum Buder found a pronounced aggregation of a *Rhodospirillum* species around 530 mμ.

For a long time these various inconsistencies have greatly puzzled me. As far as the conflicting data on the *Rhodospirillum* species are concerned, the probable solution is, nevertheless, rather simple. It is possible to reproduce all the observations so far mentioned, *viz.* the absorption spectrum found by Molisch and French, the extraction with carbon bisulfide of a red pigment which has the bacteriopurpurin β characteristics, the isolation of spirilloxanthin, and the

accumulation in a spectrum at 530 mμ, with purple spirilla. But this requires the use of two distinctly different *Rhodospirillum* species. One of these produces spirilloxanthin, has, in the living state, an absorption maximum at 550 mμ; and accordingly aggregates phototactically at this wavelength. The other, brown rather than red in cultures, does not produce spirilloxanthin, but a pigment with rhodopin-like properties. Its cultures consequently display a maximum at 530 mμ, and phototactic accumulations likewise occur at this wavelength.

It would thus be possible that Molisch also made his observations on the absorption spectrum of living bacteria with one species of *Rhodospirillum*, while using the other type for preparing the carotenoid extracts. In that case it would be understandable that he felt the need for using carbon bisulfide solutions of the red pigment in order to make the absorption spectra of living cells coincide with their extracts. This would also account for the omission in the publication of an absorption spectrum of *Rhodobacillus palustris* cells, and for the vagueness of the statement concerning the exact position of the bands.

TABLE 10

Approximate position of long-wavelength carotenoid absorption bands of different types of non-sulfur purple and brown bacteria, and of the major red and yellow pigments

GROUP OF BACTERIA	ABSORPTION BAND OF INTACT CELLS	LONG-WAVELENGTH ABSORPTION MAXIMUM OF PRINCIPAL PIGMENT IN			
		CS$_2$	C$_6$H$_6$	CHCl$_3$	EtOH
1	550	570	550	540	525–530
2	530	540–550	530	520	505
3	505	520	500	495	485

However this may be, the available information tends to favor the conclusion that the carotenoid absorption bands of the bacteria can be more nearly reproduced with benzene extracts than with carbon bisulfide extracts. It is, moreover, clear that different species of *Athiorhodaceae* which show certain well-marked absorption characteristics contain different carotenoids as principal pigment constituents. At present it is convenient to distinguish three main groups, characterized as indicated in table 10.

The pigments themselves appear to belong to the spirilloxanthin (rhodoviolascin) type for group 1, and to the rhodopin type for group 2. Those of the third group have not yet been sufficiently studied; the absorption maxima do not bear a close resemblance to flavorhodin for which the long-wavelength bands are around 500–505 mμ, 480 mμ, and 470 mμ in carbon bisulfide, chloroform, and ethanol solutions, respectively.

While in the foregoing discussion an attempt has been made to correlate the color of non-sulfur purple and brown bacteria cultures with the occurrence of certain carotenoid pigments, it should be borne in mind that it is by no means necessary that all the red and yellow pigments found in purple bacteria belong to the carotenoids. It has previously been mentioned that some strains of *Athiorhodaceae* produce a diffusible, purplish-red pigment. That this bears no

relation to the water-soluble pigment complex of the purple bacteria which French investigated and refers to as "photosynthin"[5] is shown by the following observations. In the first place, the strongly colored, bacteria-free supernatant solutions of such cultures do not, as a rule, contain proteinaceous, autolytic products which can be precipitated by the addition of acid or by heating. Even if a slight flocculent precipitate does result from such treatment, this is never more than faintly colored; by far the larger portion of the pigment remains in solution. The addition of acid does not produce the color change from red to green, described by French for "photosynthin," but a barely perceptible change to a more purplish tinge, which is entirely reversible. The solution is heat-stable, both at an alkaline and acid reaction; it can be evaporated to dryness on a water-bath without any apparent change in the pigment. Furthermore, the coloring matter is not precipitated by ammonium sulfate, even to saturation, or by trichloroacetic acid. Completely different and characteristic is also the absorption spectrum. It lacks the bacteriochlorophyll band at 590 mμ, and shows three bands, at 610, 565 and 535 mμ, of which the last is the most pronounced. These bands are not exhibited by the cells themselves.

From an alkaline solution the pigment is not extracted by a variety of organic solvents. However, upon acidification it can be readily transferred to amyl alcohol, from which it can again be extracted with an aqueous sodium bicarbonate solution. This appears to be the simplest method for purification of the dye. I have obtained it in the form of crystals but not yet in sufficient quantity to permit of further chemical tests. In old cultures of strains which produce this diffusible pigment one can frequently observe among the deposit of cells a colored precipitate which somewhat resembles these crystals, but its nature has yet to be proved.

Whatever this substance may be, it evidently is not a carotenoid. Hence one might also find other non-carotenoid pigments as products of the metabolism of the *Athiorhodaceae*. The brown to yellow pigments which are responsible for the absorption bands at 505–510 mμ of cell suspensions of certain strains thus need not necessarily be extractable with carbon bisulfide or chloroform. In this connection it is of interest to call attention to the pronounced color change, almost amounting to a bleaching, which the addition of acid produces in suspensions of these brown organisms. Such a color change is not likely to be due to a carotenoid.

Then there is the red pigment which some of the brown strains produce when growing in the presence of air. Shake cultures of such bacteria in culture tubes not covered with paraffin illustrate this phenomenon most spectacularly; the colonies in the upper few millimeters of the agar column appear a deep red, while further down light-brown to yellow colonies develop. It is apparently not necessary that the bacteria be growing in order to effect the development of the red color. A brown, liquid culture in which growth has come to a standstill will, when aerated, turn red in the course of a few hours. French (125) has shown that

[5] See also the remarks of MacKinney (126) in connection with the terminology of the pigment-protein complexes in leaves, algae, and bacteria.

the "photosynthin" extracts of brown cells do not change color upon aeration: hence the red pigment is not produced from a precursor by an auto-oxidation process. Active cell metabolism seems to be a prerequisite for the change to occur. From the absorption spectra of the aqueous extracts of brown and red cell suspensions it is evident that a new absorption band around 540 mμ characterizes the latter. Although this might suggest the formation of a pigment of the spirilloxanthin or rhodopin type, it would be premature to accept this without supporting chemical evidence. French even concludes: "Its absorption spectrum would suggest that it [is] not a carotenoid" (125, p. 408). This conclusion is based on the curve resulting from a computation of the relative absorption at different wavelengths of a brown and a red "photosynthin" extract. If, however, the yellow pigments of the brown form are involved in chemical transformations during the change from brown into red, this curve does not represent the absorption spectrum of the newly formed red component. For the present, then, this remains a problem.

The above remarks also show that the environmental conditions may considerably influence the composition of the pigment system of the purple bacteria. The most striking effects are no doubt exerted by light and by oxygen. As a rule pure cultures grown aerobically in the dark produce only little pigment, an observation made by all previous investigators. The more restricted the oxygen supply in such cases, the more pronounced is also the pigmentation. This can be seen particularly clearly on slant cultures which have been inoculated while the agar surface is yet moist. Many strains then tend to develop both on the surface of the slant and in the region between the agar and the wall of the tube, where conditions favor growth of micro-aerophilic organisms. On such slants the pigment production appears inversely proportional to the exposure of the bacteria to oxygen. The most abundant pigmentation occurs, however, in illuminated cultures, where it seems little, if at all, influenced by oxygen with the previously noted exception where a distinct color change results. Schneider's claims to the contrary must again be ascribed to his use of impure cultures resulting in a change of flora with changes in oxygen pressure and medium.

In view of the fact that oxygen does not participate in the metabolism of suspensions of non-sulfur purple bacteria in the light (p. 48) the relative independence of pigment production from oxygen tension is not surprising, although admittedly conclusions drawn from experiments with non-growing bacteria need not hold true for cultures in which multiplication takes place.

Finally, what has here been remarked concerning the effects of environmental conditions on pigment formation is based entirely on visual observations. No careful quantitative studies have yet been made on the pigments of the Athiorhodaceae. And, until the various components are better known, such investigations cannot well be attempted.

7. THE CLASSIFICATION OF THE NON-SULFUR PURPLE BACTERIA

"Für die Systematik lässt sich das Verhalten zum Licht bis jetzt wenigstens kaum verwerten; nur bezüglich der Farbstoffproduktion scheint es hin und wieder ....eine Rolle zu spielen."

Migula, (128), p. 362.

I. The taxonomic position of the group

The first official recognition which purple bacteria received as a systematic group came with the creation of the order *Thiobacteria* by Migula. Though Winogradsky on the basis of his morphological studies (11) had subdivided the sulfur bacteria into several genera, among which the greater number were composed of purple sulfur bacteria, he had not attempted to group the genera into larger units, or to clarify the systematic position of his units with respect to other bacteria.

Migula did not propose the new order till 1900. In the section on the classification of the bacteria which he wrote for Engler-Prantl's handbook (127) Winogradsky's genera of the purple sulfur bacteria occur scattered over the various families. Nor is the order *Thiobacteria* mentioned in the first volume of his masterly "System der Bakterien" (128). Between 1897, the date of publication of this volume, and 1900, when the second volume appeared (129), it must have occurred to him that the separation of the morphologically rather conspicuous sulfur bacteria from the other bacterial species was desirable. As a result the second volume of the great treatise contains the sulfur bacteria in a new order, contrasted with the order *Eubacteria* by the following definitions:

Order I. Eubacteria. "Zellen ohne Centralkörper, Schwefel und Bacteriopurpurin, farblos oder schwach gefärbt, auch chlorophyllgrün" (129, p. 1).

Order II. Thiobacteria. "Zellen ohne Centralkörper, aber Schwefeleinschlüsse enthaltend, farblos oder durch Bacteriopurpurin rosa, rot oder violett gefärbt, niemals grün (129, p. 1039).

One looks in vain, however, for an exposition of the reasons which had induced Migula to create the new orders. No doubt this omission, coupled with the great influence which Migula's "System" has exerted, is largely responsible for the perpetuation of the order *Thiobacteria* without much of an attempt on the part of later systematists to clarify the issue. The name has been changed to *Thiobacteriales* (135–137), and the order has been generally accepted. But just which organisms should constitute this group, and on what basis its members should be systematically united, these have become issues which are in dire need of careful consideration. The now existing confusion shows a regrettable lack of the application of acceptable taxonomic principles, and often a lack of interest in, or familiarity with the organisms themselves.

Yet, there is also an obvious reason for these developments which it is well to consider in some detail because a clearer view of the situation is apt to point the way to a more satisfactory solution.

In 1900 the only purple bacteria that had received special attention were the red sulfur bacteria. Winogradsky's studies on the physiology of these organisms (10, 11) had revealed a similarity to that of the colorless sulfur bacteria, both groups being capable of oxidizing hydrogen sulfide to sulfuric acid. The hitherto strictly ecological group of the sulfur bacteria had thereby been characterized more adequately on a physiological basis. Migula had used the morphological adjunct of the metabolic activity, the storage of sulfur droplets, as a criterion for his new order, and then proceeded to subdivide the *Thiobacteria* into two families.

The *Beggiatoaceae* were composed of the colorless, the *Rhodobacteriaceae* of the red or purple sulfur bacteria. What cannot be too strongly emphasized is that, at this time, all the known sulfur bacteria, both red and colorless forms, presented morphological features which seemed to set them clearly apart from the members of the Eubacteria.

Thus Molisch, in 1907, found that a family status had already been assigned to those purple bacteria which are at the same time sulfur bacteria. And in his opinion this group was closely related to the organisms of which he had made a special study. The relationship appeared, in fact, so distinct that he felt justified in combining all the purple bacteria into one new order, and in separating the colorless and the purple sulfur bacteria. *His reasoning is apt, and, if later systematists had taken better notice of it, a more satisfactory development might have been expected.* On account of the importance of the passage, it is here cited:

"Als wesentlicher Charakter würde den Thiobakterien die merkwürdige Eigenschaft zugeschrieben, in ihrem Innern Schwefel einzulagern, oder wenn man die von Nathansohn neu entdeckten nuch dazu zählt, wenigstens ausserhalb des Zellenleibes reinen Schwefel zur Abscheidung zu bringen. Die von mir entdeckte Gruppe von Purpurbakterien scheidet aber überhaupt nicht Schwefel ab, diese Fähigkeit geht ihnen, obwohl sie sonst in den physiologischen und morphologischen Eigenschaften mit den schwefelhaltigen Purpurbakterien vielfach übereinstimmen, gänzlich ab. Ich wäre daher dafür, dass man die Purpurbakterien von den *farblosen Schwefelbakterien* als *eigene Ordnung* abtrennt, hauptsächlich deshalb, weil sich auf Grund meiner Untersuchungen herausgestellt hat, dass eine grosse Zahl von Purpurbakterien Schwefel überhaupt nicht einlagern kann, also dem wesentlichen Charaktermerkmal der Thiobakterien gar nicht entspricht.
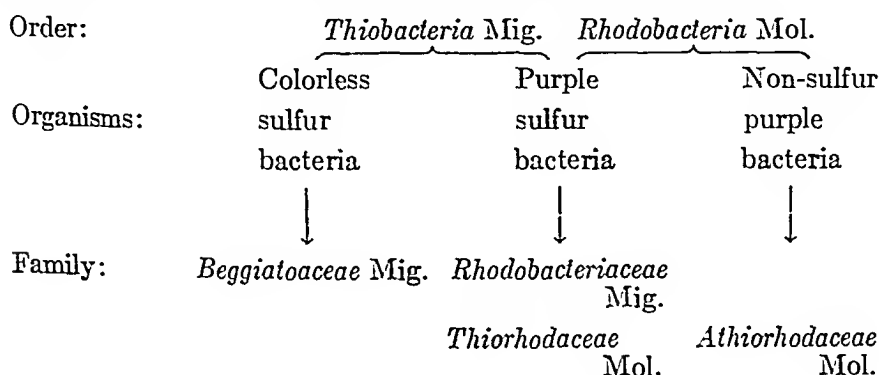
Die Purpurbakterien enthalten . . . . zwei ungemein charakteristische Farbstoffe, das rote Bakteriopurpurin und das grüne Bakteriochlorin. Und so wie man bei der systematischen Gliederung der Algen den Farbstoffen eine grosse Wichtigkeit zuerkennt, ich erinnere nur an die Phykochromaceen, Florideen und nndere, so erscheint es mir auch speziell bei den Purpurbakterien, die infolge der Lebensweise, ihres Vorkommens und ihrer Farbstoffe eine ziemlich gut umschriebene physiologische Gruppe bilden, zweckmässig, sie alle wegen ihrer eigentümlichen Farbstoffe zusammenzufassen. Ich bin mir wohl bewusst, dass sich gegen eine solche Abgrenzung vom systematischen Standpunkt Bedenken erheben lassen, zumal da ja die *Rhodobakterien* morphologisch vielfach voneinander abweichen und mit anderen bekannten farblosen Gattungen, abgesehen von dem Farbstoff, übereinstimmen.

Würde man die Purpurbakterien auf Grund ihrer morphologischen Merkmale, *die ja* bei der systematischen Sonderung die erste Rolle spielen müssen, allein gruppieren, so würden sie sich über das ganze Bakteriensystem verteilen. Vorläufig scheint mir eine physiologische Gruppierung ungemein zweckmässig, ich stehe also, in dem ich unter Purpurbakterien nicht eine natürliche Gruppe von Organismen verstehe, sondern eine physiologische, auf demselben Standpunkte wie Winogradsky bezüglich der Schwefelbakterien.

Von diesem Gesichtspunkte ausgehend, fasse ich sämtliche bisher bekannten Purpurbakterien zusammen zur Ordnung der Rhodobacteria, die sich wieder in zwei Familien gliedert, 1. in die Familie der Thiorhodaceae und 2. in die der Athiorhodaceae. Die erste umfasst diejenigen Purpurbakterien, die in ihrem Innern freien Schwefel in sichtbaren Kügelchen einzulagern vermögen, die 2. umgreift hingegen alle die Purpurbakterien, denen die erwähnte Fähigkeit vollständig abgeht" (4, p. 25–27).

With this second introduction of a large systematic unit, again created exclusively on physiological grounds, a peculiar situation arose. It is understandable

that investigators who took notice of Molisch's publication felt inclined to agree that the highly characteristic pigmentation of the purple bacteria might be as good a criterion for the foundation of an order as is the production of the special pigments which typify the large groups of the red and brown algae. But was there any good reason for disregarding the physiological similarities between the colorless and the red sulfur bacteria as a strong token of the relation between these two groups? At the time the purple sulfur bacteria appeared as a link between two utterly distinct groups of bacteria, and in the two proposals for the orders *Thiobacteria* Migula and *Rhodobacteria* Molisch these organisms formed a bone of contention. The following diagram brings this out.

Order:	*Thiobacteria* Mig.		*Rhodobacteria* Mol.
Organisms:	Colorless sulfur bacteria	Purple sulfur bacteria	Non-sulfur purple bacteria
	↓	↓	↓
Family:	*Beggiatoaceae* Mig.	*Rhodobacteriaceae* Mig.	
		Thiorhodaceae Mol.	*Athiorhodaceae* Mol.

But while Migula, unaware of the existence of a group of non-sulfur purple bacteria, could conveniently omit a consideration of its systematic position, Molisch, in stressing the obvious relationship between the two kinds of purple bacteria, might have indicated the fate of the colorless sulfur bacteria in a system of classification in which only the purple bacteria were united into an order. This he failed to do.

Since both propositions could be defended, it is only natural that systematic attempts in later years have favored a grouping of the organisms either on the basis of the sulfur metabolism with maintainance of Migula's order (especially Bavendamm (34)), or on the basis of pigment production (Richter (130); Orla Jensen (131); Benecke (132)).

The prototype of a new approach is the rudimentary classification proposed by Kruse (133), who suggested the combination of various kinds of bacteria in a separate unit "Phycobacteria."

"Manche gewöhnlich zu den Bakterien gestellte Wesen, die Beggiatoen and andere farblose Schwefelbakterien, ferner die roten Schwefel- und Purpurbakterien, die Leptothrix, Cladothrix, Phragmidiothrix, Gallionella usw.,....stehen den Spaltalgen .... noch näher. Wir möchten vorschlagen, sie geradezu Phykobakterien (Algenbakterien) zu nennen, von ihnen aber als besondere Unterordnungen die farblosen Schwefelbakterien und Purpurbakterien abzutrennen" (133, p. 1160).

The colorless sulfur bacteria and all the purple bacteria here appear as equivalent suborders. It is a little difficult to understand why the non-sulfur purple bacteria are included with other groups as providing evidence for the closer

relation with the algae; they do not possess any of the special characteristics which Kruse enumerates in support of this thesis, such as size, pleomorphism, the presence of a central body, and motility without flagella. Vahle's studies (134) had shown that, apart from the pigment production, an essential difference between the purple bacterium *Spirillum rubrum* and the colorless *Spirillum volutans* cannot be detected. It is probable, however, that Kruse was, more than Vahle, inclined to adopt Molisch's view of the relation between sulfur and non-sulfur purple bacteria, so that any argument which was built upon a consideration of the sulfur bacteria would necessitate the inclusion of the *Athiorhodaceae*.

For classification purposes Kruse's proposal was, however, too vague to be more than suggestive. Its influence may be apparent in later attempts, but since Kruse himself never introduced definite names for orders, families, etc., it is hard to trace. Nevertheless, the idea of establishing a large systematic group on the basis of general characteristics indicative of a relation of certain bacteria to algae, molds, protozoa, etc., was to be developed with a distinct appreciation for its applicability to a better system of classification, especially in the United States. It was Buchanan (135) who, after a survey of the fundamental characteristics of numerous bacterial species, concluded that as many as six major groups could be recognized for each of which he proposed an ordinal rank. The second of these orders, named *Thiobacteriales* in accordance with the rules adopted for botanical nomenclature, corresponds in part with Migula's *Thiobacteria*, and was based essentially on the work of Winogradsky:

"The work of Winogradsky and others on the sulfur bacteria has led some authors to recognize the true bacteria (*Eubacteria*) and the sulfur bacteria (*Thiobacteria*), as primary coordinate groups" (135, p. 160).

Yet, the delimitation of the group differs in one respect quite markedly from that of Migula. Buchanan wrote:

"The thiobacteria [are] characterized by certain relationships to sulphur. They all grow best in the presence of hydrogen sulphid, and always contain sulphur granules or bacteriopurpurin or both" (135, p. 161).

In the publication here referred to Buchanan did not mention Molisch's publications on the purple bacteria. This is included in the bibliography to a later paper of the series (136) which contains a definition of the order:

"*Thiobacteriales* Ordo nov.

Cells various, typically containing either granules of free sulphur, or bacteriopurpurin, or both, usually growing best in the presence of hydrogen sulphide. The cells are plant-like, not protozoan-like, not producing a pseudoplasmodium or a highly developed encysted resting stage. Spores are rarely or never formed" (136, p. 461).

Hence, whereas Migula had considered the presence of sulfur globules as an obligatory criterion for all members of his order *Thiobacteria*, this now has become a facultative characteristic, equivalent to, and apparently replaceable by the presence of bacteriopurpurin. It seems obvious that this emendation was inspired by the recognition of the group of non-sulfur purple bacteria, and by Molisch's contention of the close relationship of *Thio-* and *Athiorhodaceae*. Since Molisch had also demonstrated that the last-mentioned organisms are not sulfur bacteria in Winogradsky's sense, the earlier description ("they all grow best in the presence of hydrogen sulphid") had to be modified accordingly.

Here, then, we see the three groups, the colorless sulfur bacteria, the purple sulfur bacteria, and the non-sulfur purple bacteria, formerly in part combined into two overlapping orders by Migula and Molisch, merged into one systematic unit. Buchanan's suggestion was adopted by the committee of the Society of American Bacteriologists (138), commented upon favorably by Breed, Conn, and Baker (139), and it has been incorporated in the past 5 editions of Bergey's Manual of Determinative Bacteriology. Also Pribram (140) accepted the order in the above sense. Very similar is, furthermore, the system proposed by Bavendamm (151) in 1936. The points of difference are, however, worth a brief discussion because they emphasize a difficulty which will presently be taken up.

In introducing his classification Bavendamm states: "In Anlehnung an den verdienstvollen Erforscher der Schwefelbakterien Winogradsky, der diesen Namen zuerst aufgestellt hat und durch van Niel insofern gerechtfertigt ist, als tatsächlich die Verwertung des Schwefels und seiner Verbindungen als einziger Energiequelle das feste Bindeglied zwischen allen Thiobakterien ist, bin ich nicht dem Vorschlag von Engelmann und Molisch gefolgt, den farblosen Schwefelbakterien die Purpurbakterien entgegenzusetzen. Ich habe vielmehr den Leuco- und Chlorothiobakterien die Rhodothiobakterien zugestellt und die schwefelfreien Purpurbakterien zunächst den schwefelspeichernden anhangsweise zugeordnet" (151, p. 48).

So definite is here the stress on the sulfur metabolism that this becomes the sole criterion of the group of Thiobacteria:

"Bakterien, die Sulfide, Sulfite, Thiosulfate und elementaren Schwefel dehydrieren. Es sind entweder farblose oder gefärbte, obligate oder fakultative Anorgoxydanten, die Schwefel und seine Verbindungen als einzige Energiequelle verwerten können" (151, p. 49).

The subdivision of this group is then carried out as follows:

A. Colorless sulfur bacteria, chemoautotrophic (*Leucothiobacteria*).
B. Colored sulfur bacteria, photoautotrophic (*Chromothiobacteria*).
    I. Purple sulfur bacteria (*Rhodothiobacteria*); with addendum: non-sulfur purple bacteria (*Athiorhodobacteria*).
    II. Green sulfur bacteria (*Chlorothiobacteria*).

The *Athiorhodobacteria* do not, apparently, constitute a group of equivalent rank with the *Leuco-*, *Rhodo-*, and *Chlorothiobacteria;* they form an appendix to the second, with the following characterization:

"Durch Bakteriopurpurin rot gefärbte Bakterien, meist von üblicher Bakteriengrösse, die offenbar Übergangsformen der Purpurschwefelbakterien darstellen. Sie sind nicht in der Lage, Schwefel und seine Verbindungen als einzige Energiequelle zu verwerten. Sie sind obligat heterotroph, aerob im Dunkeln und anaerob im Licht" (151, p. 49).

This passage shows that Bavendamm cannot but admit the very close relationship between sulfur and non-sulfur purple bacteria; in fact, the latter are considered as transition stages. But how can one ever hope to properly place one of these organisms, specifically described as obligatory heterotrophic, unable to use sulfur and its compounds as sole energy sources, in a larger group for which the autotrophic nature and the oxidation of inorganic sulfur compounds is a prerequisite?

Obviously, in so emphasizing the "sulfur bacteria" nature of the *Thiorhodaceae*, the sulfur-free counterparts must be either left out, or smuggled in. If the two groups of purple bacteria are considered as taxonomically related, this should come out in the definition of the larger unit of which they both form a part. This Bavendamm failed to do, and hence his method of treatment is clearly inferior to that of Buchanan, although the latter's diagnosis of the *Thiobacteriales* is

open to the criticism that it is difficult to understand why the presence of bacteriopurpurin is somehow equivalent to the occurrence of sulfur globules.

Admittedly, the synthesis accomplished by the establishment of Buchanan's order appears fully justifiable since the purple sulfur bacteria seem to form such an obvious link with the colorless sulfur bacteria on the one hand, and with the non-sulfur purple bacteria on the other. But there is a fallacy in this argument which has not been adequately realized. This is the exclusive use of two completely independent characters, occurring together in one type, for establishing relationships between groups which each possess only one of them. In this way it would be argued that because there are organisms which cause both an alcoholic fermentation and a plant disease, the group of bacteria which produce alcohol must be related to those which have a different metabolism but are plant pathogens. It is, of course, not restricted to the use of physiological characteristics; any two independent characters combined in one, and also found separately in other groups would lead to the same conclusion. The argument is logically inadmissable.

Consequently it becomes necessary to re-evaluate the evidence for the affinities between the colorless sulfur bacteria, the purple sulfur bacteria, and the non-sulfur purple bacteria, so that the order *Thiobacteriales* as now defined may be replaced by a more acceptable grouping of the organisms that comprise it.

Firstly we should face the question whether the facts justify the maintenance of a systematic unit based upon a specialized sulfur metabolism. Winogradsky's investigations (10, 11), restricted to the larger forms, gave the impetus to its creation, both in view of the peculiar morphology of the "sulfur bacteria," and of the important new principle in biochemistry (chemo-autotrophism) which he derived from his physiological studies. But the work of Nathansohn (141), Jacobsen (142, 143), Beijerinck (144, 145), Lieske (146), Waksman and Joffe, (147), Starkey (148), and others made it evident that the same fundamental type of metabolism, the oxidation of hydrogen sulfide, sulfur, and thiosulfate, is shared by many organisms which are morphologically typical *Eubacteriales*. To be sure, these do not, as a rule, deposit sulfur droplets inside their cells, and hence do not belong to the "sulfur bacteria" as long as the latter are defined as organisms internally containing recognizable globules of elementary sulfur. Such a definition is, however, quite unsatisfactory, as has been pointed out before. (Cf. p. 3; also (3, p. 56–57)). Those who have examined the typical microflora of sulfur springs are familiar with the organism which Miyoshi (149) named *Leptothrix sulfurea*, and which differs from *Thiothrix* only in that the filaments do not seem to store sulfur inside, but deposit it externally. Since there are good reasons for believing that the cell diameter determines the location of the sulfur deposition, it is much more reasonable to base the definition of a sulfur bacterium on its characteristic metabolism. In that case one would, however, have to include the *Thiobacillus* species, etc., whereby the group would become so heterogeneous that its usefulness seems problematical. Such an assemblage certainly could no longer be considered as even remotely "natural."

This must have been responsible for the attempts at formulating the group

of "sulfur bacteria" in such a manner as to avoid the need for including the genus *Thiobacillus*. But despite the fact that this has been achieved, however artificial and unsatisfactory the manner, the present group of "genuine" colorless sulfur bacteria is, upon closer inspection, no more than a conglomerate of species whose only claims to inclusion in a common order seem to be that they contain sulfur globules, and are difficult to fit into any of the remaining orders. It hardly needs pointing out that a group which contains the colorless counterparts of *Oscillatoria*, *Phormidium*, and *Schizothrix*, as well as the large, non-flagellated *Achromatium*, the flagellate *Thiophysa*, and the vibrio-like *Thiospira*, corresponds so little to a systematic entity that even the inclusion of *Thiobacillus* would not make it less satisfactory.

This leads to the conclusion that the colorless sulfur bacteria had best be abandoned as a group, except perhaps in a physiological sense. In the latter interpretation there is no reason to exclude the pseudomonas-like *Thiobacillus* species. For taxonomic purposes the various members should be distributed over a number of quite distinct and separate groups.

If this be accepted as a logical procedure, then the present order of the *Thiobacteriales* becomes limited to the purple bacteria, in fact corresponds to Molisch's order *Rhodobacteria*, or Pringsheim's *Rhodobacteriales* (150). Once regarded as a physiological group, characterized by the presence of bacteriopurpurin, it can today be defined more adequately since it has been shown that this pigment—or at least its green component—permits of a photosynthetic metabolism. In the previous sections it has been observed repeatedly that this metabolism is fundamentally similar in both the sulfur and the non-sulfur purple bacteria, and that the photosynthetic aspects mark it as quite distinct from that of other bacteria, including the colorless sulfur bacteria. Is this a strong enough argument to maintain a systematic unit for all the purple bacteria?

It appears that different investigators have been inclined to answer this question in different ways. Schneider, the first to take up this matter after the photosynthetic nature of the metabolism of the purple bacteria had been established, wrote:

"Im Gegensatz zu Bavendamm (1924) glaube ich, dass man bei einer Beurteilung der Verwandtschaft dieser Bakteriengruppen nach phylogenetischen Gesichtspunkten die Thiorhodaceen mit den Athiorhodaceen zusammenfassen und sie nicht wegen ihrer Fähigkeit zur Speicherung von Schwefel den farblosen Schwefelbakterien zuordnen sollte" (22, p. 83).

As we have seen, Bavendamm later did decide to adopt a system of classification in which the two groups of purple bacteria were kept close together. But because he still clung to the idea of a major group of "sulfur bacteria," the attempt led to considerable difficulties.

Fundamentally different was the solution which Pribram (152) proposed in 1933. It represents one of the few modern attempts to develop a classification which is consistently based upon the use of morphological characteristics only. Hence the various purple bacteria, both *Thio-* and *Athiorhodaceae*, are found scattered over three of the four orders of the subclass *Algobacteria*, along with

colorless species of *Micrococcus, Pseudomonas*, etc. This is not the place to enter into a detailed discussion of Pribram's system. It may suffice to state that, on account of the very limited number of morphological features used, it contains much that seems of doubtful value. Few microbiologists to-day would, I believe, want to take too seriously an arrangement in which *Chromatium, Pseudomonas,* and *Myxococcus* are placed in Winslow's family *Pseudomonadaceae*, while *Serratia* and *Hillhousia* together form a second family of the *Pseudomonadales*, and in which the sulfur purple bacterium *Rhodonostoc* is considered as being linked with the *Streptococcus* species by *Leuconostoc mesenteroides.*

The primary use of morphological criteria was also adopted by Kluyver and van Niel (153) in developing an outline for the classification of the *Eubacteriales*. Here, however, physiological characteristics were considered as equally indispensable, though they were employed only for the definition of genera. Since morphologically the various purple bacteria do not exhibit features which are different from those of the "true bacteria," they were all included in this order, forming the genera with a photosynthetic metabolism (153, table 1). In this way a clear-cut separation of the purple bacteria from the nonphotosynthetic *Eubacteriales* is achieved. The only drawback is that the group as a whole is not represented by a single taxonomic unit. This is due to the fact that the purple bacteria cannot be defined as a group except on the basis of their unique photosynthetic metabolism. As long as certain orders, families, and tribes of bacteria are segregated on morphological grounds, then obviously these customary taxonomic terms cannot be used to designate groups of organisms with the same general morphology, but with special physiological properties.

Perhaps this is not too serious a disadvantage. By combining morphological and physiological characters for the delimitation of smaller systematic units (genera) a system is developed in which similarities in one or the other aspect can easily be visualized. Table 11, a greatly condensed version of the one published by Kluyver and van Niel (153), makes this clear.

Such a tabulation shows that the use of important morphological characters as the guiding principle in bacterial classification results in a columnar arrangement of genera which may together be regarded as higher taxonomic units (e.g. families), whereas the primary use of physiological properties leads to rows of genera possessing this attribute. The decision as to which of the two shall be used for the creation of larger systematic entities may seem quite arbitrary. And, in fact, the two alternatives have been used in the past without much discrimination. One fact stands out clearly; wherever they have been employed side by side in the same system the outcome has been confusing. Only by a consistent use of one or the other can this be avoided.

But is the selection merely a matter of personal preference, of a desire to stress the aspects in which the particular investigator has been most interested? I believe that there are good reasons for thinking otherwise. Stanier and van Niel (154) have shown that at the present time one can clearly distinguish three primary classes among the bacteria on the basis of their general morphology. These, the *Eubacteriae*, the *Myxobacteriae,* and the *Spirochaetae,* represent groups

without any clearly recognizable interrelations, but in themselves they appear to be quite homogeneous. Again, in the *Eubacteria* definite assemblages stand out more or less clearly as morphologically characterized entities (153). It is true that there exist a number of organisms whose affinities are not yet very distinct, but this does not affect the main thesis.

On the other hand, some of the outstanding physiological properties that have been deemed of a sufficiently essential nature to be used for the purpose of combining larger groups of bacteria occur scattered throughout a number of morphological "families." In many cases it is quite obvious that such physiological groups are far less uniform than the morphological ones. Good examples of this kind are the sulfur bacteria, the hydrogen bacteria, the iron bacteria, the urea bacteria, etc.

TABLE 11

Morphological and physiological characteristics of some of the genera of the Eubacteriales

PHYSIOLOGICAL TYPE OF METABOLISM	MORPHOLOGICAL		
	Polarly flagellated rods	Spherical organisms	Peritrichously flagellated rods
Photosynthetic	*Chromatium, Rhodovibrio, Rhodospirillum*, etc.	*Thiopedia, Thiosarcina*, etc.	
Chemoautotrophic	*Sulfomonas, Nitrosomonas*, etc.	*Nitrosococcus, Siderocapsa*, etc.	
Heterotrophic oxidative	*Pseudomonas, Vibrio, Spirillum*, etc.	*Micrococcus, Sarcina*, etc.	*Kurthia, Bacillus*, etc.
Fermentative	*Zymomonas*, etc.	*Streptococcus, Betacoccus*, etc.	*Aerobacter, Aerobacillus, Clostridium*, etc.

These considerations tend to favor the view that a physiological classification is less apt to lead to a rational system as far as the larger units are concerned than a strictly morphological one. The columns rather than the rows in table 11 would thus appear the more logical for consolidation into groups of higher taxonomic rank. Naturally, special names for physiological groups will continue to be used. However, they should not be converted into family or order designations. Sometimes a physiological character may be associated with a morphologically restricted group. The "lactic acid bacteria" are a good case in point. Nevertheless, one must not lose sight of the fact that the term "lactic acid bacterium" generally implies a morphological as well as a physiological characterization. If all the bacterial types capable of fermenting sugars with the formation of lactic acid as the chief metabolic product were included, the group would certainly not represent a taxonomically acceptable unit.

The above arguments for a primarily morphological classification apply, of course, to the purple bacteria. Nevertheless, in this instance one could make out a good case for the use of a physiological property. The photosynthetic ability of this group of organisms could well be considered as a characteristic of such far-reaching importance that it would seem adequate as the basis for a major differentiation in the bacterial kingdom. It may here be reiterated that a separation of the bacteria from the bluegreen algae is at present impossible in any other way (See 154). It could even be contended that such a segregation of the purple bacteria would rest on a morphologically detectable difference provided by the occurrence of a special pigment system. This, however, would be valid only on account of its established function; past attempts to differentiate bacterial groups larger than genera by pigmentation have invariably led to difficulties.

In any case, even the use of the photosynthetic metabolism for the creation of an order for the purple bacteria in the class *Eubacteriae*, as was proposed by Stanier and van Niel, is not free from objections. While for the moment photosynthesis may appear to be an utterly distinctive process, future developments in our understanding of the photosynthetic process may well obliterate this. When first conceived, the distinction between auto- and heterotrophic metabolism seemed quite sharp indeed; during the past several years it has become increasingly evident that a clear line of demarcation cannot be drawn (59).

The purple bacteria so far known form a group in which there is but a limited morphological diversification. More in particular is this true for the non-sulfur purple bacteria; they all fall, as has been pointed out, in the morphological family of the *Pseudomonadaceae*, and represent the pseudomonas, vibrio, and spirillum types, resembling completely the non-photosynthetic members of these tribes. If, under certain conditions, one of the non-sulfur purple bacteria would fail to produce its prominent pigment system, it would thereby become indistinguishable from a typical *Pseudomonas*, *Vibrio*, or *Spirillum* species. The loss of chlorophyll formation in certain genera of algae is well known, and the parallel genera *Chlorella-Prototheca*, *Euglena-Astasia*, *Chlamydomonas-Polytoma* among the *Chlorophyta*, as well as *Oscillatoria-Beggiatoa*, *Phormidium-Thiothrix*, *Schizothrix-Thioploca* in the *Myxophyta* show convincingly the derivation of the colorless forms. Since the transformation from pigmented to non-pigmented forms, but not the opposite, has been experimentally achieved, it appears more logical to consider the purple bacteria as the progenitors of the corresponding non-photosynthetic bacteria.

This does not imply that each and every one of the representatives of the latter group should have an immediate counterpart among the purple bacteria. It is to be expected that various modifications of colorless forms would have arisen secondarily. But an independent derivation of the purple bacteria and the bacteriochlorophyll-free representatives of the spherical and polarly flagellated *Eubacteriae* would necessitate the assumption of a fully parallel development in the two groups. The segregation of the purple bacteria as an order in the

Eubacteriae, as proposed by Stanier and van Niel (154), would suggest this less probable relationship.

For these reasons it seems to me preferable to abandon also the physiological group of the purple bacteria as a taxonomic unit, and to incorporate the various members as genera in the corresponding morphological families of the *Eubacteriae.* The primary subdivision of this large assemblage can then be carried out much more consistently; the order *Rhodobacteriales* could profitably be replaced by an order *Pseudomonadales,* comprising all polarly flagellated, gram negative bacteria.

By thus proposing a taxonomic revision in which neither the order *Thiobacteriales* Buchanan, nor the order *Rhodobacteriales* Pringsheim is maintained, a grave inconvenience from the point of view of determinative bacteriology is likely to result. Heretofore the purple bacteria as well as the colorless sulfur bacteria, both possessing rather outstanding characteristics which made their allocation to a restricted group a simple matter, could be identified much more readily than would be possible on the basis of a rigidly morphological system of classification. This situation can be greatly improved by realizing that the restriction of Latin names for the columns in Kluyver and van Niel's diagram pertains exclusively to the problem of taxonomy. This does not mean, however, that additional sets of determinative keys could not be prepared which would allow of the rapid differentiation of bacteria on the basis of physiological characteristics. This, in fact, seems a most desirable elaboration of the systems now in use which are practically without exception based upon a confused utilization of morphological and physiological criteria for the differentiation of orders and families. If this were done, the accumulated experience of many workers in different branches, and with varied methods of approach could be made available in a much more accessible manner than is possible by the present compromises. Complications can be avoided by the use of common, non-Latin names for such physiological groups, i.e., for the conglomerates of organisms which are represented by the rows in the before-mentioned diagram.

Past criticism of both "scientific" and "utilitarian" systems have frequently failed to recognize the need for both approaches. There has been an unfortunate tendency to identify a proposed system of classification with a set of determinative keys. The adoption of a number of accessory keys for determinative purposes would, I believe, materially simplify the problem of achieving a more consistent system of classification without sacrificing its usefulness from the determinative standpoint.

If, for example, a key were available by which various "sulfur bacteria"—but not *Thiobacteriales* or *Rhodobacteriales*—could be identified as far as the genus, then such an ecological-physiological group, comprising *Thiobacillus, Beggiatoa, Chromatium* species, etc., would not be in the least objectionable, since this group does not now constitute a larger, real or pretended, *taxonomic* unit. Yet it would be of considerable help to the student of an ecological community of microörganisms who has had no opportunity or desire to familiarize himself with representatives of other groups. Also certain families, e.g. the *Nitrobacteriaceae, Lactobacteriaceae,* etc., could more logically be replaced by common designations (chemo-autotrophs, acetic acid and lactic acid bacteria), and thus become more recognizably what they, indeed, are: physiological groups.

Since a determinative system, at least as far as the keys are concerned, is aimed at being avowedly utilitarian, an approach from as many angles as are now represented by specialized interests could in this manner be consolidated. At the same time, the final "system of classification" would not need to be a compromise. Even organisms of uncertain systematic position, temporarily placed in one or more appendices to the "system," would still be "determinable" with the aid of one or more of the various keys.

The allocation of the non-sulfur purple bacteria to an order *Pseudomonadales* of the *Eubacteriae* appears to me taxonomically desirable, as pointed out before. The genera, discussed in detail in the next section, belong morphologically in the families *Pseudomonadaceae* and *Spirillaceae*. Hence the once united group of the "*Athiorhodaceae*" becomes scattered, though, as will shortly appear, not to the extent which Molisch feared as a result of abandoning an attempt at creating a "physiological order" for the purple bacteria. With the aid of determinative keys, including aspects of the physiology and ecology of the organisms, the purple and brown bacteria can, however, still be admitted as a special group on the basis of their pigment system and photosynthetic metabolism. But the characterization of this group will then be divorced from implications of primary taxonomic significance.

II. *Detailed classification of the non-sulfur purple and brown bacteria: the genera*

There exists only one detailed systematic treatment of the non-sulfur purple and brown bacteria based upon original observations. This is the system developed by Molisch (4) in which the family *Athiorhodaceae* is subdivided into seven genera, each one morphologically defined. Since a very small number of morphological characteristics appeared sufficient for a differentiation, the resulting system has the irresistable appeal of simplicity. This can best be appreciated by an examination of Molisch's arrangement which follows.

Family *Athiorhodaceae*.
a. Cells combined into families.
    Division in one direction only.
       1. Cells rod-shaped, many embedded together in a common mass of slime
                                                                   Rhodocystis.
       2. Cells spheres or short rods, arranged like strings of beads, each string surrounded
          by a slime capsule   *Rhodonostoc.*
b. Cells single.
    Division in one direction.   *Rhodococcus*
       1. Cells spherical, immotile   *Rhodobacterium.*
       2. Cells straight rods, immotile   *Rhodobacillus.*
       3. Cells rod-shaped, motile
       4. Cells short, curved, bean- or comma-shaped, with a polar flagellum, very motile
                                                    Rhodovibrio.
       5. Cells spirally wound, with polar flagellum or tuft of flagella   *Rhodospirillum*

This system has stood the test of time remarkably well. For 35 years it has been copied without a single essential modification by every one who has written on this group of organisms. The only change has been based on nomenclatorial considerations. Since the generic name *Rhodococcus* appeared to be a homonym, Buchanan, in 1918 (136), proposed the substitution of *Rhodosphaera* for *Rhodo-*

coccus. Seven years later the same author (137) pointed out that also the new name "is invalid as a generic designation for the bacteria, as the name *Rhodosphaera* was given by Engler in 1881 to a genus in the family *Anacariaceae* among the flowering plants" (137, p. 449). Hence in the second and all subsequent editions of Bergey's Manual of Determinative Bacteriology it was replaced by *Rhodorhagus*.

Unquestionably the general tendency to accept Molisch's system so completely is due to the fact that so few investigators have afterwards studied this group of purple bacteria at first hand. Furthermore, the descriptions are clear, in spite of their brevity, and their value is tremendously enhanced by the excellent photomicrographs representing the seven genera. Even a superficial acquaintance with the various members of the group often suffices to identify a culture as *Rhodocystis, Rhodonostoc, Rhodobacillus,* or *Rhodospirillum*. The recent descriptions, published by Czurda and Maresch (20), of some representative strains appear, in comparison, far from satisfactory. Their paper would have benefited greatly from the inclusion of illustrations.

Because five of the seven genera are represented by a single species, while in *Rhodococcus* only two, and in *Rhodospirillum* three species were recognized by Molisch, the determination of a genus is practically equivalent to the complete identification of a given strain of purple bacteria on the basis of his system. Also in this respect very little of importance has been added by later workers. Two additional species of *Rhodospirillum* have been proposed by Hama (50, 155) on entirely insufficient grounds. Three attempts have been made at amplifying Molisch's descriptions. The first deals with *Spirillum (Rhodospirillum) rubrum* which Molisch recognized as a purple bacterium without describing it, probably because he felt that Esmarch's characterization was satisfactory. Of this bacterium Vahle (134) published a detailed and accurate description. Both the others pertain to *Rhodobacillus palustris.* Plowe *et al.* (156) made some observations on an organism isolated from the intestines of a Cerambycid beetle which they considered identical with the above-mentioned species. Claiming that Molisch's characterization was inadequate, they published a brief description of their culture which shows unmistakably that it cannot have been *Rhodobacillus palustris,* and makes it highly probable that it was not even a purple bacterium. Schneider's account (22) of *Rhodobacillus palustris* is rendered worthless by the fact that he did not use pure cultures.

However useful Molisch's system may have been, it cannot be denied that it presents a few features which to-day distinctly limit its validity. As has previously been stated, every one of the large numbers of cultures which I have examined during the past 12 years has appeared motile. In each case a polar arrangement of the flagella has also been demonstrated. Since there are good reasons for believing that all the previously observed non-sulfur purple bacteria are now represented in my collection, this finding invalidates the description of the genus *Rhodococcus (Rhodorhagus),* and eliminates altogether the genus *Rhodobacterium.* Furthermore, it is virtually impossible to decide what type of organism is to be designated as *Rhodovibrio.* This genus was described by Molisch on the basis of observations with impure cultures. Now it is perfectly true that one may find purple bacteria which agree with the characteristics of *Rhodovibrio.* But it is equally certain that under different conditions the same strains are morphologically indistinguishable from *Rhodobacillus.* This is, perhaps, most strikingly illustrated by the fact that Molisch himself has presented evidence to the same effect. A painstaking comparison of the photomicrographs of *Rhodobacillus palustris* from a gelatin plate culture, and of *Rhodovibrio parvus* (4, Plate 1, Fig. 1, and Plate 2, Fig. 10) fails to reveal any perceptible difference between the two organisms. On several occasions I have isolated what appeared to be indubitable *Rhodovibrio* strains, but they have invariably shown a range of morphological variation which includes the most typical habitus of *Rhodobacillus palustris* (4, Plate 1, Fig. 2). *Vice versa,* I have observed with all strains of the latter organism cultures which should be designated as characteristic *Rhodovibrio* cultures.

Not having been in a position to follow the development of *Rhodovibrio parvus* for lack of a pure culture, Molisch apparently considered the morphology of what he observed in some crude cultures as sufficiently distinctive and constant to warrant the creation of this genus. On the basis of our present information it seems to me necessary to conclude that *Rhodobacillus* and *Rhodovibrio* do not actually constitute distinguishable genera.

The genus *Rhodocystis* is morphologically characterized by the formation of cell masses embedded in a common slime capsule, while *Rhodonostoc* consists of single strands surrounded by mucus. There are in my collection 25 strains of purple bacteria which, on the basis of a number of morphological and physiological properties, I consider as closely related. Some of these show the typical *Rhodocystis* clumps, and produce cultures in which single individuals or strands are extremely rare. Yet other strains of this group, when grown in liquid media, give rise to a mucilaginous sediment that can easily be redispersed. In this case one finds few clumps; the cultures consist chiefly of capsulated chains of cells or even of single individuals. Again, the genus *Rhodocystis* was created on the basis of observations on crude cultures; only *Rhodonostoc* was apparently studied in cultures derived from single colonies, as witnessed by Molisch's description of a stab culture. Although from my own experience I am fully convinced that there undoubtedly exist organisms corresponding to these genera, and which are different in several respects, Molisch's method of differentiation must be deemed much too simple.

Yet another difficulty concerning the genus *Rhodonostoc* lies in its close similarity to *Rhodococcus*. This appears conclusively from an examination of the hundreds of photomicrographs of cultures which can be recognized as belonging to either one of these genera. Depending upon the culture conditions one and the same strain may, also in this case, suggest its identity with *Rhodonostoc* or *Rhodococcus*. The close resemblance can also be inferred from Molisch's own descriptions, although his illustrations show examples of cultures which can be easily differentiated on a morphological basis. In the description of *Rhodonostoc capsulatum* Molisch calls special attention to the brown color of this organism in stab cultures, and concludes: "Der braune Stich dieser Bakterie ist recht charakteristisch." (4, p. 23). But also the representatives of the genera *Rhodocystis* and *Rhodococcus* are brown instead of red in stab cultures. This must have escaped Molisch's notice because it is only under anaerobic conditions that the brown color of *Rhodococcus* is pronounced, and he evidently did not prepare stab cultures of *Rhodococcus*.

On account of these facts it seems very doubtful whether the three genera, each with one or two species, should be maintained. They might, at least for the present, be more logically combined. There appears to be some justification for preserving the genus *Rhodocystis;* the group of cultures of this type is characterized by a number of properties which set them apart from the other brown and purple bacteria. Since, however, the most important diagnostic feature of *Rhodocystis*, viz., the occurrence of cell masses in a common slime capsule, is not shared by all the strains of this group, the name would be misleading. Also, the rather exclusive color of Molisch's *Rhodocystis gelatinosa*, referred to as "pale peach-colored," is characteristic only for some of this group of strains, and even for these not in all media. In yeast extract, for example, all strains are brown, though of various shades.

The *Rhodocystis* strains are the most outspokenly rod-shaped of the various brown bacteria. But even *Rhodococcus* develops in certain media as distinct rods. The three genera therefore possess in various degrees the morphological characters of the *Pseudomonadaceae*.

In view of the pseudomonas nature of the red organisms previously designated as *Rhodobacterium*, *Rhodobacillus*, and *Rhodovibrio*, Kluyver and van Niel have proposed the new generic name *Rhodomonas* for the species of this group (153). Czurda and Maresch have, however, pointed out (20) that this had previously been used by Karsten for a genus of cryptomonads. For this reason they replaced it by *Rhodopseudomonos*.

On similar grounds Kluyver and van Niel (153) tentatively proposed a genus *Phaeomonas* to include the different brown representatives of the non-sulfur purple bacteria. This was later accepted by Czurda and Maresch (20). As the type species for the genus the former authors designated *Phaeomonas (Streptococcus) varians* (Ewart). Unfortunately this

organism has not been characterized by its original author (19) in such a manner as to permit of its unequivocal identification. A careful appraisal of Ewart's description in the light of our present knowledge makes it even doubtful whether his *Streptococcus varians* is, indeed, a member of the purple bacteria group. Hence the genus *Phaeomonas* assumes a doubtful status. It appears, therefore, advisable to use a new generic name for which a more appropriate type species can be designated, or to dispense with the genus altogether.

The former procedure would make it possible to separate the "brown" and the "red" non-sulfur purple bacteria into groups of generic rank. But such a separation is not as easy as it might seem because a number of these brown bacteria develop with a red pigment under aerobic conditions. Even if in the diagnosis it were specified that the brown color is typical only for anaerobic cultures difficulties would still arise. I have frequently observed distinctly red cultures of certain of these strains under strictly anaerobic conditions, especially in malonate media. Furthermore, the characteristically red strains of the *Rhodobacillus palustris* type appear brown in certain media. It is clear, then, that at the present time a satisfactory differentiation would have to be based upon a completely arbitrary, and not even convenient, evaluation of color differences. A better understanding of the nature of the pigments themselves, and of their distribution in the various types may, of course, change the situation.

In view of these considerations, and also of the common morphological features of both the red and brown groups of these purple bacteria and the very small number of distinguishable species, it seems more appropriate to abandon such an attempt until more extensive data are available to suggest the desirability of recognizing more than one major group. For the time being it is consequently proposed to classify the rod-shaped and spherical to ellipsoidal non-sulfur purple bacteria as a single genus, *Rhodopseudomonas* Czurda and Maresch, with an amended diagnosis. The type species is *Rhodopseudomonas (Rhodobacillus) palustris* (Molisch).

The last genus of Molisch's system, *Rhodospirillum*, is morphologically so distinctive that little needs to be said about it. The existence of brown photosynthetic spirilla has previously (153) led to the proposal of the generic name *Phaeospirillum* for these bacteria. Since the four strains of brown spirilla which I have so far been able to study appear, apart from the color difference and a more pronounced tendency towards anaerobiosis, in all respects very similar to the red ones, such a generic segregation seems premature also in this case. At present I can see no objection to classifying all the photosynthetic spirilla as members of the single genus *Rhodospirillum* Molisch, with the type species *Rhodospirillum rubrum* (Esmarch) Molisch.

By thus reducing the number of genera of Molisch's family *Athiorhodaceae* to only two, the difficulties inherent in his system disappear. Still, this limitation to two genera is not entirely satisfactory. The fact is that the differences between e.g., *Rhodobacillus palustris* and *Rhodospirillum rubrum* do not appear larger nor more significant than between the former and *Rhodococcus capsulatus*. However, as has already been pointed out, the typically spherical *Rhodococcus*, itself occasionally occurring as rods, is linked to the characteristically rod-shaped purple bacteria by *Rhodonostoc* in such a manner that as yet it is too difficult to draw even an arbitrary line of demarcation which would result in a more acceptable system.

As definitions for the two genera the following are proposed.

Genus *Rhodopseudomonas* Czurda and Maresch. Emend.
Spherical and rod-shaped bacteria, motile by means of polar flagella. Non-sporeforming, gram negative. Contain bacteriochlorophyll which enables them to carry out a photosynthetic metabolism. The latter is dependent upon the presence of extraneous oxidizable substances, and proceeds without the evolution of molecular oxygen. Though some members are capable of oxidizing inorganic substrates, none is strictly autotrophic. Produce accessory pigments causing the cultures, especially in the light, to appear in various shades of brownish yellow to deep red.

The type species is *Rhodopseudomonas palustris* (Molisch). The genus includes the members of Molisch's genera *Rhodobacterium*, *Rhodobacillus*, *Rhodovibrio*, *Rhodocystis*, *Rhodonostoc*, and *Rhodococcus*, as well as the genera *Rhodosphaera* Buchanan, *Rhodorhagus* Bergey *et al.*, *Rhodomonas* Kluyver and van Niel, *Phacomonas* Kluyver and van Niel, and *Rhodopseudomonas* Czurda and Maresch.

Genus *Rhodospirillum* Molisch.
Spiral-shaped bacteria, motile by means of polar flagella. Non-sporeforming, Gram negative. Contain bacteriochlorophyll, and are potentially photosynthetic in the presence of extraneous oxidizable substances. Molecular oxygen is not produced. Unable to live in strictly mineral media. Cultures in the light are brown to red, due to the formation of accessory pigments.

The type species is *Rhodospirillum rubrum* (Esmarch) Molisch. The genus includes *Phacospirillum* Kluyver and van Niel.

It will be noted that no mention is made of the absence of sulfur globules in the cells; the separation from the sulfur purple bacteria rests upon the ability of the latter to develop strictly autotrophically. It is conceivable that some *Thiospirillum* (*Thiorhodospirillum*) species, none of which has so far been isolated or even studied with respect to their metabolism, may turn out to require organic substances. If this were the case, such species would be included in the genus *Rhodospirillum* as here outlined. I see no objection to this consequence.

A few words must yet be said about the brown bacteria reported by Utermöhl (18). Describing a remarkable combination (*Pelochromatium roseum* Lauterborn?) of small brown bacteria surrounding a larger organism, the author stated:

"Der Hauptgrund für meinen Zweifel, ob die vorliegende Form wirklich zu den Purpurbakterien gehört . . . . ist die Farbe, die durch ihr mattes braunrot auffällig von den mehr rosa- bis violettroten Farbtönen der übrigen Purpurbakterien absticht . . . ." (18, p. 606). "Aus Mangel an Zeit habe ich keine weiteren, mikrochemischen Untersuchungen vorgenommen, aus denen die Art der Farbstoffe näher zu ersehen wäre. Doch dürfte aus meinen Beobachtungen hervorgehen, dass es sich bei den Hüllbakterien um eine neue, den Purpur- und Chlorobakterien vergleichbare Reiche farbstoffführender Bakterien handelt, die ihrer braunen Färbung wegen wohl als Phaeobakterien bezeichnet werden können" (18, p. 607). "Das Binnenbakterium möchte ich als *Endosoma palleum*, die Hüllbakterien nach dem Entdecker dieses Zellverbandes als *Lauterborniola minima* bezeichnen" (18, p. 609).

The description is extremely cursory and so incomplete that it is not even possible to decide whether *Lauterborniola* has a definite connection with the brown members of the photosynthetic bacteria. Utermöhl qualified his first publication as a preliminary com-

munication; the only later reference I have been able to find occurs in an extensive paper by the same author on the phytoplancton of the lakes of Eastern Holstein (157). Here, however, the section on Phaeobacteria occupies only a single paragraph, with the remark:

"Über diese Formen habe ich bereits an anderer Stelle ausführlich berichtet (reference to (18)), so dass ich mich hier sehr kurz fassen kann."

Until further studies will have clarified the nature of *"Lauterborniola"* it is, therefore, necessary to omit it from the various genera now included in *Rhodopseudomonas*.

III. Detailed classification and description of the non-sulfur purple and brown bacteria: the species

"For in all analysis it is the business of the analyser to get at the ultimate unities; when he has reached the ultimate unities it is also his business to respect them: further division will show acuteness, but it will not show judgment."
Hillaire Belloc "On the simplicity of words". (160, p. 23)

In the previous section it has been pointed out that the morphological characteristics on which Molisch based six of his seven genera of *Athiorhodaceae* do not suffice for a satisfactory differentiation of the organisms. It is true that a thorough familiarity with the various representatives, such as comes with a prolonged study of the group, will enable one to recognize special types which correspond more or less closely to some of Molisch's species. Nevertheless, the information now available concerning the morphology and physiology of a large number of strains should permit of a description of such types in a more immediately useful manner.

Observations on the morphological properties of well over 150 strains, cultured in a large variety of media and under different conditions, have shown that none of the organisms can be adequately characterized by describing its size and shape in a single culture. In the course of time permanent slides, mostly nigrosin mounts, have been prepared of all cultures that seemed of interest from the point of view of keeping a record of these studies. Dr. Arthur L. Cohen has made close to 2000 photomicrographs of these slides, which have made it possible to compare accurately the behavior of individual isolates. The following descriptions are based partly on this material.

Furthermore, the interpretation of the basic physiological properties of this group has led to a study of the growth of each one of the strains in the presence of 40 different, simple substrates. The technique used for these experiments has already been described (cf. p. 33–36). The results have revealed that there exist a number of close correlations between the morphological types and the utilizability of a variety of compounds. Since a large number of strains of each type was available for these studies, the range of physiological variation within each group can also be indicated with a fair prospect of being more or less representative in this respect.

A survey of these morphological and physiological data indicates that six groups of non-sulfur purple bacteria can readily be distinguished. Four of these belong to the genus *Rhodopseudomonas* as previously defined: the remaining two comprise the spiral-shaped organisms, united in the genus *Rhodospirillum*.

On the whole, the various strains which constitute one such group form a rather

homogeneous assemblage. Its members have a number of properties in common which permit of their allocation by means of a variety of morphological, physiological, and biochemical criteria. It seems, therefore, fully justifiable and expedient to consider each such group as a species.

I realize that it would be possible to stress individual strain differences so as to provide for a larger number of species. However, such a procedure is not at present advisable inasmuch as the significance of the differences cannot yet be evaluated in association with other aspects of the organisms. Especially in the absence of extensive observations on the constancy and variability of most of the physiological and biochemical characteristics of any one strain it seems more logical to adopt a policy in which the species are limited to groups with a number of correlated properties. I am wholeheartedly in agreement with the conclusion, reached by Parr and Robbins (161): "It is obvious that our results favor the view that classification should be conservative. New species should only be recognized on the basis of thoroughly well-worked-out cultural differences having real significance, and, if possible, correlated with other characters."

If, later, the need for a further differentiation arises, it is obvious that the reasons prompting it can be more readily incorporated in the manner of subdivision as long as details of uncertain value have not previously been used for the classification of these organisms.

The four groups representing the genus *Rhodopseudomonas* correspond, as far as can be ascertained, rather closely with the species *Rhodobacillus palustris*, *Rhodocystis gelatinosa*, *Rhodonostoc capsulatus* and *Rhodococcus capsulatus*, all of them described by Molisch. They will be designated as *Rhodopseudomonas palustris*, *Rhodopseudomonas gelatinosa*, *Rhodopseudomonas capsulatus*, and *Rhodopseudomonas spheroides*. Molisch's species *Rhodococcus minor*, *Rhodovibrio parvus*, and *Rhodobacterium capsulatum* seem to be, at least to-day, unrecognizable entities.

The fifth group, comprising the *Rhodospirillum* strains, presents a similar problem. Also here two subgroups can readily be segregated. They are composed of the red and the brown photosynthetic spirilla respectively. Since, in the strains I have studied, the pigmentation is correlated with other distinct morphological differences, the recognition of at least two separate species is indicated. But in addition, the various isolates of the red spirilla show unmistakable evidence of further mutual differences. There is, consequently, some reason for increasing the number of species. Here again, with but one exception of a morphologically outstanding type, I have not actually been able to decide, on the basis of the available data, how a further splitting up of the remaining group of red spirilla should be carried out. This group has, therefore, been considered provisionally as a single species, and future investigations will have to show the way in which a more satisfactory treatment of this group is to be achieved in case a need for a continued subdivision will be felt.

In the following description of the species use has been made of those morphological and physiological characteristics which appear to me as significant, both from the point of view of identification and differentiation. A number of commonly recommended descriptive characters have been omitted because they are either in no way characteristic for any one

species, or because they have appeared so variable and dependent upon environmental conditions that no useful purpose is served in mentioning them. This latter case applies, for example, to the shape and size of colonies. Also, certain of the routine procedures for the study of pure cultures of bacteria have not been included in the present investigation since they did not *a priori* offer much promise that information of a basic nature could thereby be obtained. It is granted that, by so doing, the descriptions are "incomplete." But the same may be said of the published accounts of those bacterial species whose only characterization is based upon the use of standardized techniques. It is not uncommon to find that such descriptions convey a rather misleading impression concerning the general nature of the organisms and their outstanding physiological functions, even though some arbitrary tests may serve the useful purpose of permitting a rapid differential diagnosis. An outstanding example of this has been cited by Otto Rahn (158) when he pointed out that, merely on the outcome of the routine tests, *Hydrogenomonas pantotropha* would become indistinguishable from *Phytomonas vascularum*. This may simply mean that a more complete investigation of *Hydrogenomonas pantotropha* with respect to its possible plant pathogenicity, and of *Phytomonas vascularum* as a potential hydrogen oxidizing bacterium would actually establish their identity. In view of the recent claims (Elrod and Braun, 159) that *Phytomonas polycolor* would be the same organism as *Pseudomonas aeruginosa* this would hardly be surprising. It only serves to bring out the fact that the ordinarily practiced description of a bacterium is not necessarily the most satisfactory, nor can it lay claim to any degree of completeness. It is to be anticipated that each particular group of microorganisms can best be studied by some special methodology which would be of little use elsewhere.

These introductory remarks may suffice to justify the approach here used for the following descriptions of the species of non-sulfur purple bacteria. Since the generic diagnoses given in the preceding section cover certain common features, these will not again be mentioned.

<div align="center">

Rhodopseudomonas palustris

Synon.   *Rhodobacillus palustris* Molisch.

Rhodovibrio parvus Molisch.

Rhodomonas palustris Kluyver and van Niel.

</div>

This species is represented by 48 individual strains, obtained at various times by a number of different methods. Included are isolations from surface water and mud samples in Holland, California, and Cuba. The Cuban mud samples were made available through the kindness of Dr. J. Heath under whose supervision they were collected, and who forwarded them to me. They have been invaluable in permitting me to test the general validity of the specific enrichment culture methods with material from a 'far removed locality. Three strains were isolated from impure cultures, kindly furnished by Drs. E. Schneider, Breslau, and H. Gaffron, then at Berlin-Dahlem.

Morphology. (figs. 1–3; 18–26) The bacteria are usually distinctly rod-shaped, although in certain media, and especially in young cultures, very short, vibrio-like organisms may predominate. The size is extraordinarily variable, even for one and the same strain, and is strongly influenced by the age of the culture and by the composition of the medium. The most consistently short cells have been observed in young yeast extract cultures, especially when incubated anaerobically in the light (figs. 1, 26), or in media in which development is generally slow and scanty, such as in the basal medium with malonate (figs. 18, 21, 24). They then measure about 0.6–0.8×1.2–2μ, and often appear slightly curved. More frequently the cells are considerably longer, and may attain a length of 10μ.

Highly characteristic is the pronounced tendency to the formation of rather

irregularly shaped, bent and crooked, long rods, occasionally swollen at one or
both extremities, and frequently suggesting branching· These forms can always
be found in older cultures, where they occur as clusters, strikingly reminiscent of
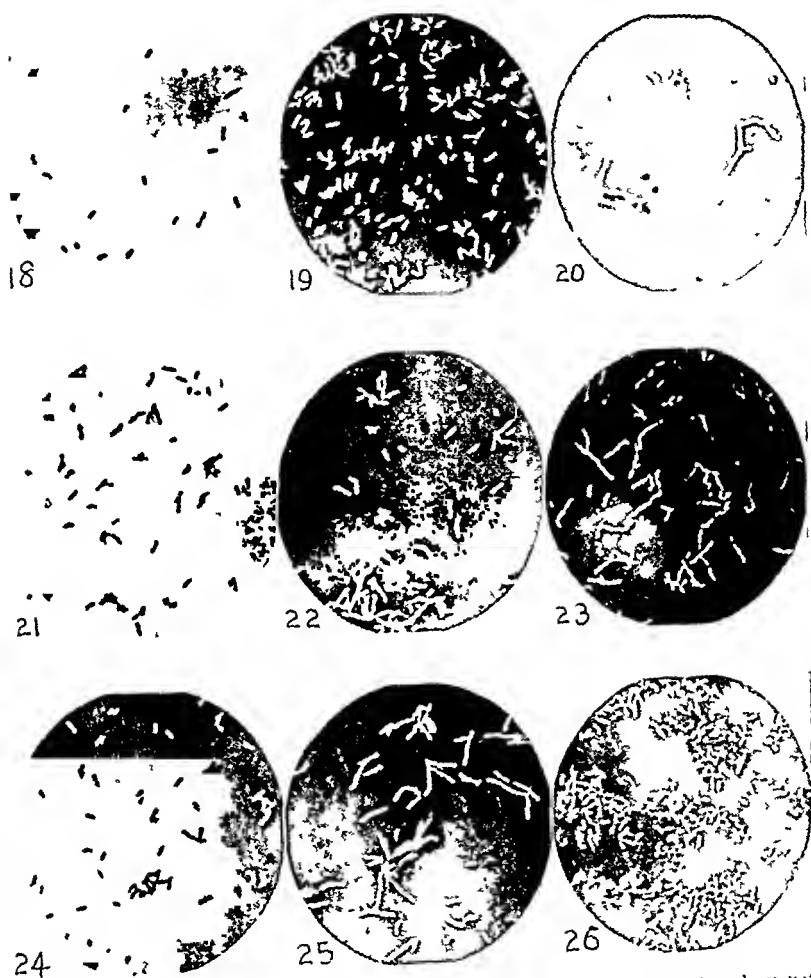
FIGS 18-26   *Rhodopseudomonas palustris*, grown anaerobically in basal medium with
various substrates, × 800

18-20	Strain No 52, with		
18		0 2%	Na-malonate
19		0 2%	Na·S₂O₃
20		0 1%	Na-n-caproate
21-23	Strain No 66 with		
21		0 2%	Na-malonate
22		0 2%	Na-crotonate
23		0 2%	Na-propionate
24-26	Strain No 82 with		
24		0 2%	Na-malonate
25		0 05%	Na-n-caprylate
26		1%	yeast autolysate

Corynebacterium and *Mycobacterium* species. Such cells are, as a rule, immotile. This behavior is the most readily distinguishing morphological feature of the species (figs. 3, 20, 23, 25).

Growth in liquid media is never mucoid; the sediment which is deposited as the cultures grow older appears homogeneous and smooth, and can readily be redispersed.

Color. The pigment production leads to cultures varying from a light pink to a dark brownish red. This again depends upon the medium; where development is slight, as in malonate, thiosulfate, and usually, glycerol, the lighter shades predominate, while in media containing fatty acids the cultures become more nearly brown.

Most strains produce a water-soluble pigment which tinges the supernatant liquid of older cultures a clear red.

Physiology. The development in yeast extract is not markedly influenced by the reaction of the medium over a range from pH 6 to 8.5. With fatty acids as the main substrate the combined effect of low pH and relatively high fatty acid concentration (0.1–0.2%) may prevent growth. The temperature optimum is generally rather high, good development being possible at 37°C. In this respect there exist, however, strain differences, somewhat lower temperature optima being exhibited by isolates which have been maintained in pure culture for a long period of time.

Characteristic odors are not observable, save that old cultures may develop a faint ionone-like fragrance.

Most strains are able to grow on the surface of agar plates; a few are considerably more sensitive to oxygen and develop only in stabs, in which the upper region may remain free of growth. This behavior is capable of being changed, apparently through adaptation; some strains which behaved like true anaerobes when first isolated are now uninhibited by atmospheric oxygen tension.

None of the strains liquefies gelatin, as shown by gelatin yeast extract stabs kept under observation for as long as 60 days.

Biochemical characteristics. Outstanding among the biochemical characteristics, as revealed by the comparative studies in the basal medium with 40 simple organic compounds (See Chapter 5, Section II), are the ability to grow in thiosulfate, and the rapid and profuse development in glutarate and ethanol media. All strains have failed to grow in solutions in which mannitol, sorbitol, glucose, or mannose were supplied as hydrogen donors; if the media contain an additional, utilizable hydrogen donor development is normal, so that the carbohydrates do not appear to be inhibitory.

Though not all strains have been tested as to their ability to oxidize molecular hydrogen, those which have been so examined have given positive results.

Of the six amino acids used in this investigation (glycine, alanine, leucine, asparagine, aspartic and glutamic acids) only leucine has invariably given positive cultures; only four strains have grown feebly with alanine, six with glutamic acid, three with asparagine, and two with aspartic acid.

Distinguishing features of this species are its morphological resemblance to

Mycobacterium species, its ability to grow with thiosulfate as oxidizable material, and its inability to attack mannitol, sorbitol, and the carbohydrates.

Enrichment cultures. In accordance with the general biochemical characteristics of the species, it is easily obtained by enrichment cultures, using the basal medium with ethanol, glutarate, or thiosulfate. The last-mentioned solution will, of course, give rise to the simultaneous development of purple sulfur bacteria. For this reason it is simplest to start cultures with alcohol or glutarate. If other non-sulfur purple bacteria are present in such abundance that the isolation of *Rhodopseudomonas palustris* presents difficulties—which, in my experience, has never been the case—subcultures in thiosulfate medium will eliminate the other species almost entirely.
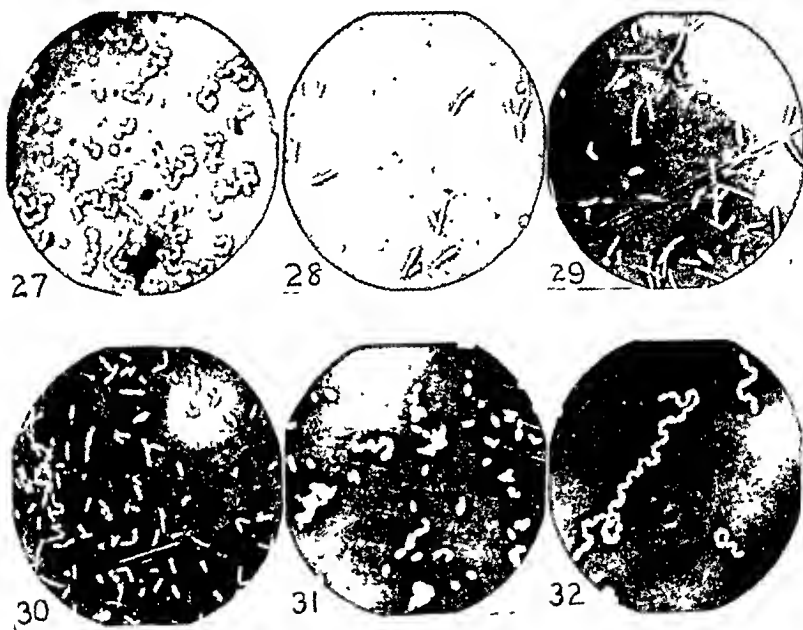
FIGS. 27–32 *Rhodopseudomonas capsulatus*, grown anaerobically in basal medium with various substrates; × 800
27. Strain No 49 with Na-*i*-caproate
28. Strain No. 102 with Na-lactate
29. Strain No. 26 with Na-propionate
30 Strain No. 26 with Na-acetate
31. Strain No. 42 with glucose
32. Strain No. 44 with fructose

Rhodopseudomonas capsulatus

Synon. *Rhodonostoc capsulatus* Molisch.

Only 16 strains of this species are present in my collection They have been isolated from enrichment cultures with various organic acids as substrates, and using mud samples from different parts of California, and from Cuba. The 16 representatives show a remarkably uniform behavior.

Morphology (figs. 4–6; 27–38). Depending upon the pH of the medium, the organisms occur as motile, nearly spherical cells, or as distinct rods. The former

are found in media with a pH below 7; they often form chains, and may thus present some resemblance to streptococci (figs. 27, 32, 33, 34). The rod-shaped bacteria are characteristic for media at a pH above 7, and the higher the pH, the longer are also the rods (figs. 28–30). The individual cells usually measure slightly less than 1 μ in width, though in an alkaline environment (pH 8 or above) the rods are attenuated, and often not more than 0.5 μ wide (fig. 30), while in the presence of glucose or fructose slightly swollen individuals can be observed (1.2 μ; figs. 31, 32). The length of the organisms ranges from 1 to 6 μ; in neutral
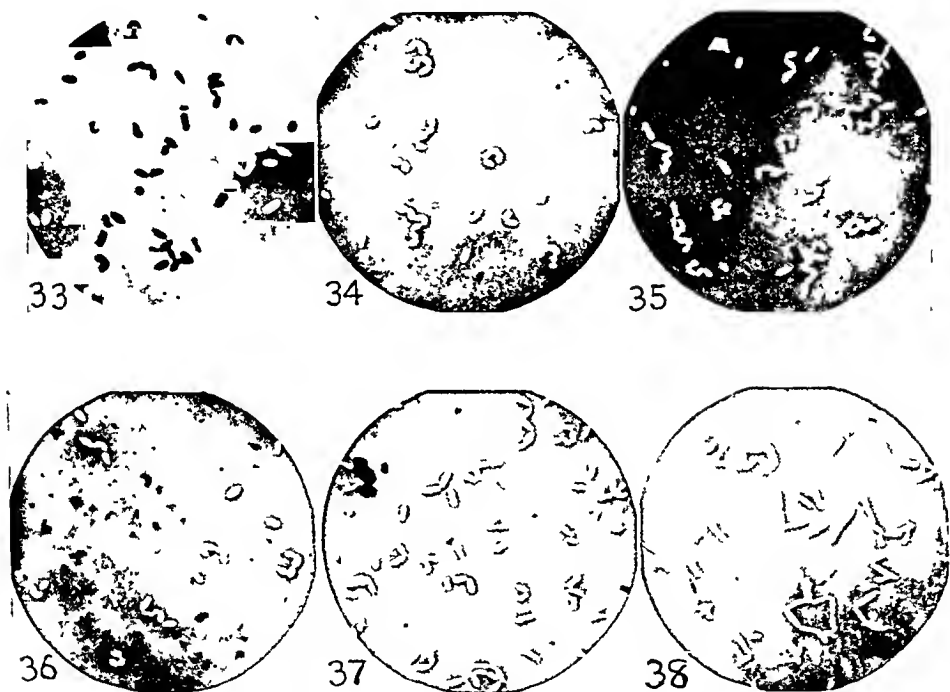
FIGS. 33–38. *Rhodopseudomonas capsulatus.* grown anaerobically in basal medium with various substrates; × 800.
33 Strain No. 44 with Na-i-butyrate
34. Strain No. 44 with Na-i-valerate
35. Strain No. 44 with Na-lactate
36. Strain No. 102 with Na-malonate
37. Strain No. 34 with Na-propionate
38 Strain No. 27 with Na-i-butyrate

to slightly alkaline solutions the most frequent size is 2 to 2.5 μ. At pH 8 or above an irregular, filamentous and quite abnormal growth occurs.

Special mention should be made of the zigzaggy arrangement of the cells in chains; the angular aspect of the latter is so outstanding a feature that on this alone an identification can be based (figs. 33–38). Cultures in media at pH 8 or above are slimy.

Color. Practically all anaerobic cultures appear brown, the shade ranging from a light, yellowish-brown to a deep mahogany brown. Only in malonate media, where development is not abundant, do the organisms produce a reddish-

brown growth. With none of the strains has the formation of a water-soluble red pigment been observed.

In the presence of oxygen, both in aerobic liquid cultures and on agar plates, additional pigments are produced; the growth under these conditions is distinctly red.

Physiology. Also this organism can develop in yeast extract media over a pH range from at least 6 to 8.5, although at the higher pH the growth is morphologically abnormal. The available strains show a decidedly lower temperature optimum than those of *Rhodopseudomonas palustris*; none develops at a temperature above 30°C.

Most cultures are practically odorless; occasionally a very faint peach-like flavor has been detected.

Growth is not inhibited by the presence of oxygen.

Gelatin liquefaction has never been observed.

Biochemical characteristics. Of the 40 substrates tested, the fatty acids and many of the substituted acids are good carbon sources. All strains grow rapidly and abundantly in propionate media. Glutaric acid leads, at best, to very meager cultures; tartrate, citrate, and gluconate are not utilized. Also ethanol and glycerol, as well as mannitol and sorbitol, fail to produce growth. Glucose and fructose, on the other hand, appear to be satisfactory substrates, while mannose is not attacked. Further diagnostically valuable properties are the ability of all strains to grow in alanine and glutamic acid media, while they fail to do so with leucine.

Thiosulfate is not oxidized; a few strains have been found capable of oxidizing molecular hydrogen, the others have not yet been tested in this respect.

Distinguishing properties. While the most useful morphological criteria are the brown color of anaerobic cultures and the cell-shape of the organisms, especially the appearance of the chains, *Rhodopseudomonas capsulatus* can also be readily distinguished from other species by its ability to grow with propionate, glucose, fructose, alanine, and glutamic acid, and by the absence of development with the above-mentioned alcohols, and with mannose, leucine, and thiosulfate.

Enrichment cultures. The organism can usually be obtained from enrichment cultures with any one of a number of organic acids, particularly propionic, lactic, and succinic acids. Frequently this species also predominates in peptone or yeast extract media. Since the latter are, however, always badly contaminated with a large number of non-photosynthetic bacteria, isolation may be greatly facilitated by one or more transfers to a propionate medium with around 0.2% propionate. By streaking from successful bottle cultures on yeast or peptone agar plates and incubation under aerobic conditions in the dark, the elimination of some other purple bacteria can be accomplished.

The utilizability of glucose and fructose cannot, of course, be made the basis of a satisfactory enrichment method. There are too many non-photosynthetic bacteria which can develop anaerobically with these sugars. Under their influence the substrate is rapidly transformed into a number of decomposition products before the *Rhodopseudomonas gelatinosa* cells have become numerically preponderant. Meanwhile, the initial medium is changed so as to be no longer specific for any one member of the non-sulfur purple bacteria

Rhodopseudomonas spheroides

Synon. *Rhodococcus capsulatus* Molisch
        Rhodococcus minor Molisch
        Rhodosphaera capsulata Buchanan
        Rhodorhagus capsulatus Bergey *et al.*
        Rhodosphaera minor Buchanan
        Rhodorhagus minor Bergey *et al.*

A total of 19 strains with a number of common characteristics has been singled out as representatives of this species. They have been obtained in Delft, Holland, and in California from a variety of enrichment cultures, using different media and inocula.

Morphology (figs. 7, 8; 39–54). The bacteria of this group are the most nearly spherical of any of the non-sulfur purple bacteria. In young cultures actively motile, they lose their motility with age, especially if the medium becomes alkaline. The size of the individual cells is extremely variable; without capsule they measure from 0.7 to as much as 4 μ in diameter (figs. 39–50). In media which are or become very alkaline, irregular, swollen and distorted rods are produced, having the appearance of involution forms (figs. 49; 51–54); sugar-containing solutions frequently give rise to egg-shaped, ovoid cells, the dimensions of which generally vary from 2 x 2.5 to 2.5 x 3.5 μ (figs. 45, 50). Although such cells look much more "normal" than the before-mentioned "involution forms" it should be pointed out that in cultures of one and the same strain numerous intermediate shapes have been found between slender, rod-shaped organisms, the ovoid cells, and large, bizarre forms, 2.5μ wide, and often attaining a length of more than 10 μ (figs. 49–54).

The individuals regularly occur singly, only rarely does one encounter short chains.

Considering the variable size of these bacteria it becomes clear that the recognition of species within this group cannot be based solely on cell dimensions, as practiced by Molisch for the separation of *Rhodococcus capsulatus* and *Rhodococcus minor*. Since furthermore the physiological and biochemical characteristics of the strains are quite homogeneous, I have deemed it desirable to recognize for the present only a single species.

Color. As in the case of *Rhodopseudomonas capsulatus*, the anaerobic cultures of *R. spheroides* are brown in color, varying in shade from a light dirty greenish to dark brown. With but four exceptions all strains are capable of producing a water-soluble red pigment with the characteristic absorption maxima of 535 and 565 mμ. When grown in the presence of oxygen the organisms are distinctly red.

Physiology. Again the pH range over which development is possible is wide, and extends from at least pH 6 to 8.5. The temperature optimum is relatively low, and lies below 30°C.

All cultures exhibit an unpleasant, putrid odor; those in which the pH has risen to 7 or above are stringy, due to abundant mucus production.

None of the strains liquefies gelatin.

Biochemical characteristics. Cultures of this group in the basal medium with various substrates show, as a rule, much less copious development than those of

the previous two species. Because better results are obtained by increasing the amount of yeast extract in the medium it seems likely that the growth-factor
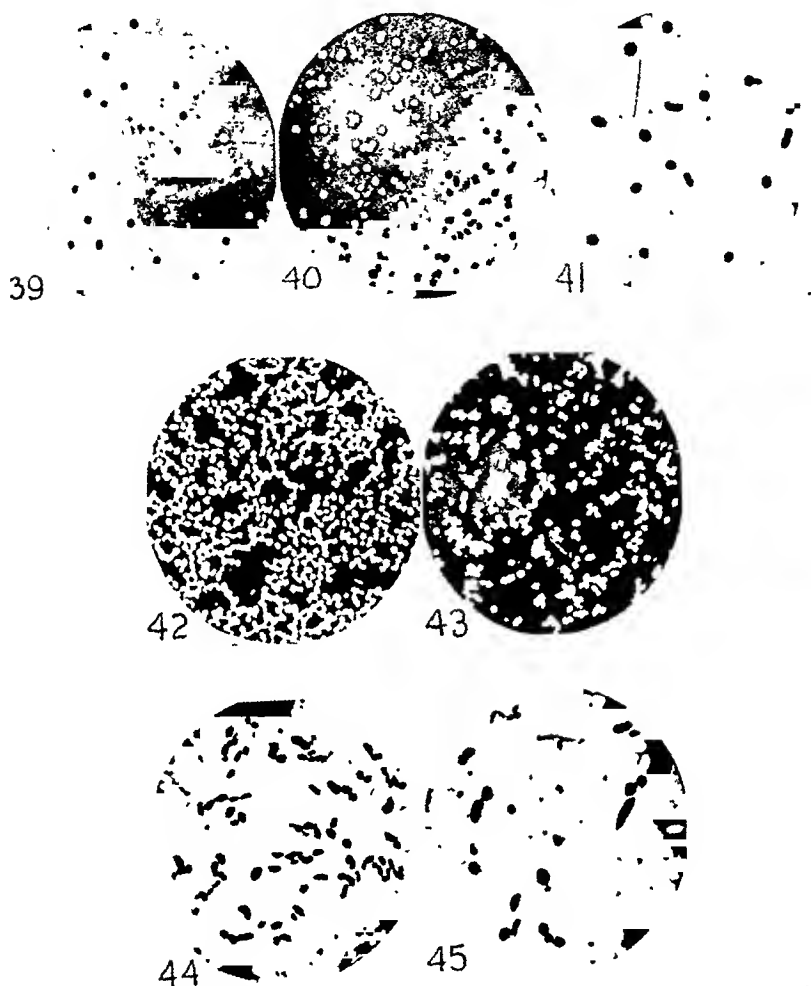
FIGS. 39–45. *Rhodopseudomonas spheroides*, grown anaerobically in basal medium with various substrates; × 800.
39. Strain No. 106 with yeast autolysate
40. Strain No. 106 with Na-aspartate
41. Strain No. 106 with Na-malate
42. Strain No. 33 with yeast autolysate
43. Strain No. 33 with Na-crotonate
44. Strain No. 33 with sorbitol
45. Strain No. 33 with fructose

requirements of *Rhodopseudomonas spheroides* are greater than those of *R. palustris* and of *R. capsulatus*.
Also the strains are much more adversely affected by fatty acids; growth in

media with the normally used concentrations of propionic. caproic, or pelargonic acid has never been obtained.
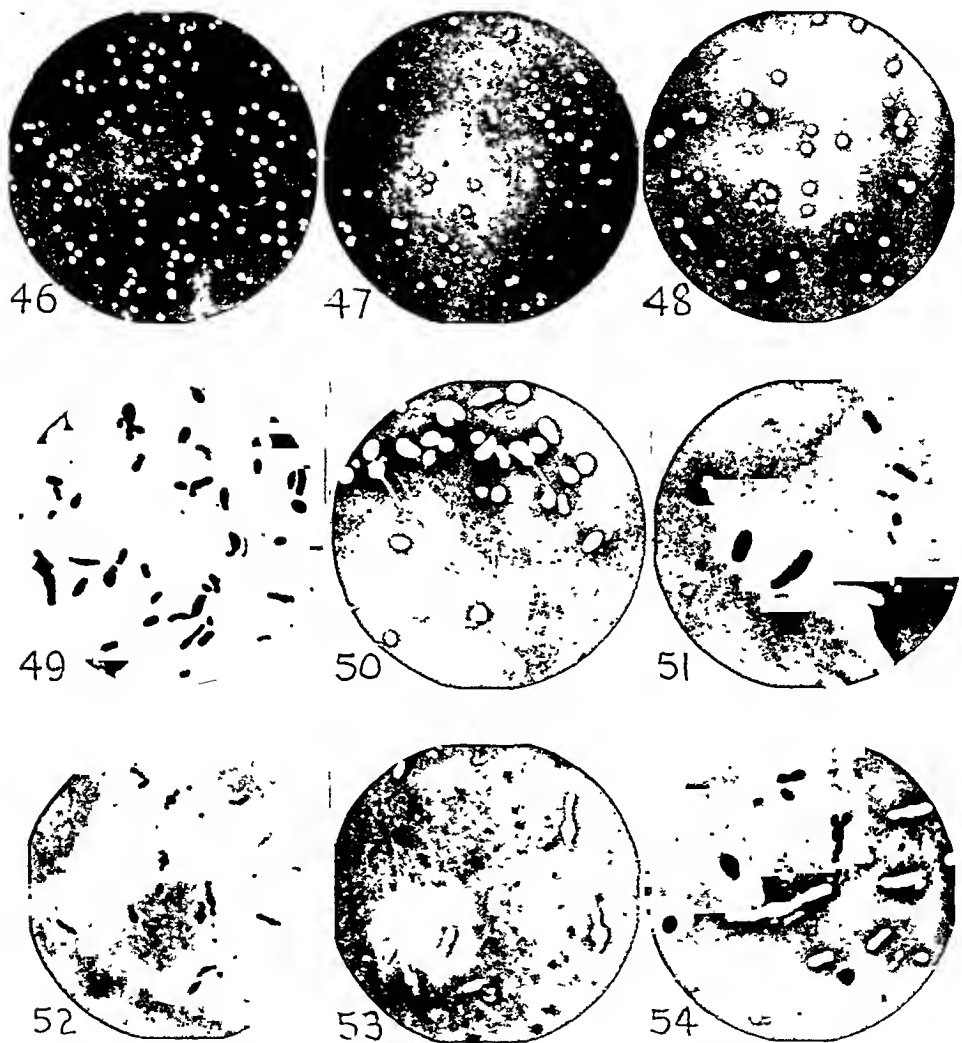

Figs. 46-54. *Rhodopseudomonas spheroides.* grown anaerobically in basal medium with various substrates; × 800.

    46. Strain No. 80 with Na-succinate
    47. Strain No. 80 with ethanol
    48. Strain No 80 with Na-*n*-valerate
    49. Strain No. 80 with Na-*n*-caproate
    50. Strain No 80 with glucose
    51. Strain No 80 with Na-*n*-butyrate
    52. Strain No 48 with yeast autolysate. pH 8 5
    53. Strain No 28 with   "   "   pH 9
    54. Strain No 29 with Na-malonate

All of the members of this group have developed in media with tartrate, gluconate. ethanol. glycerol. mannitol. sorbitol. glucose. fructose. and mannose.

Growth in glucose media is accompanied by acid production. The pH of such media may decrease to 4.0 before development stops. The acidic decomposition of glucose occurs both in the presence and in the absence of air, and in light as well as in darkness. In illuminated cultures the acid tends to disappear later on. None of the amino acids used has given consistent results; only two strains have shown growth in alanine, 5 in asparagine, 7 in aspartic and glutamic acids, and 9 in leucine. Correlations have not been observed, however. One of the strains growing slightly with alanine also produced a meager culture with leucine, while the other developed in the presence of aspartic acid.

Growth on the basis of thiosulfate oxidation has not been observed; and the three strains which have been investigated with respect to their ability to oxidize hydrogen have given negative results.

Distinguishing characteristics are, in addition to the typical cell-shape, and the color of the cultures, especially the growth in tartrate and gluconate, in ethanol, glycerol, mannitol, sorbitol, glucose, fructose, mannose, and the failure to grow with thiosulfate.

Enrichment cultures. Since, for the reasons given above (p. 94), glycerol, mannitol, sorbitol, glucose and mannose are not suited as substrates for the direct enrichment of this species from mud or water samples, the best method is based upon a combination of a number of its characteristics. Sometimes it is possible to isolate pure cultures from enrichment cultures with methanol, ethanol, and other alcohols, with fatty acids, or with tartrate. Excellent results are in general obtained by transferring crude cultures in alcohol media to tartrate, by which procedure *Rhodopseudomonas palustris*, the more abundant species in the former medium, can gradually be eliminated while at the same time insuring an inoculum in which purple bacteria are present in far greater numbers than colorless organisms which can be enriched with tartrate in the absence of air (27). Streaks on yeast agar plates, incubated aerobically in the dark, readily lead to pure cultures.
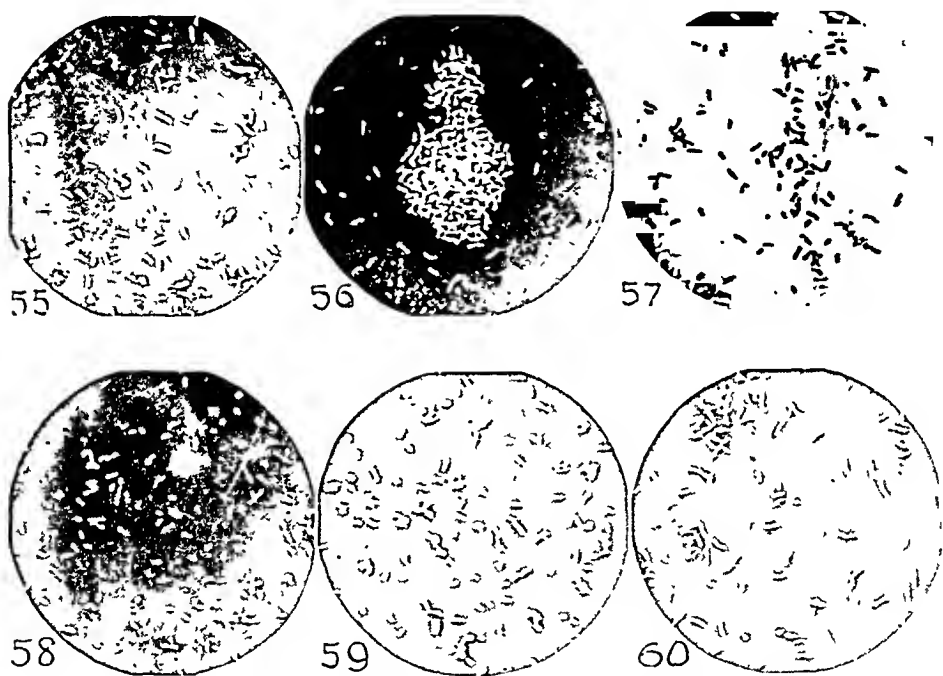
Rhodopseudomonas gelatinosa
Synon. *Rhodocystis gelatinosa* Molisch

The description of this species is based upon the study of a group of 24 strains with a number of common characteristics. All of them have been isolated in California, from a variety of mud samples, including some from Cuba.

Morphology (figs. 55–66). In young cultures the cells appear as short and small rods, approximately 0.5 μ wide, and 1 to 2 μ long (figs. 55–60). They are actively motile, but this is often difficult to ascertain because of the very extensive mucus production which causes the individuals to clump together (See p. 15). This smallest of the *Rhodopseudomonas* species displays, like the others, a striking morphological variability. Most characteristic are the cell shapes found in old cultures; they are much longer, at times swollen, and appear as irregularly curved rods, up to 15 μ long and in places 1 μ wide (figs. 64–66). In this stage the cells bear some superficial resemblance to those of old *R. palustris* cultures, but the typical mycobacterium-like clusters of the latter are here replaced by more irregularly curved conglomerates.

Since the cultures are extraordinarily slimy, individual bacteria are only observable when a thorough homogenizing procedure, such as shaking with glass beads, is practiced. Frequently even slides made from such material still reveal a certain degree of orientation of the elements (fig. 56).

Color. Of diagnostic significance, which cannot yet be associated with any specific pigments, is the color of anaerobic cultures in most liquid media. It has already been likened to the color of a culture of sulfur purple bacteria in which the cells are stuffed with sulfur; Molisch used the designation "peach color." It is quite pale, distinctly pinkish, and delicate. Only in yeast extract media, where growth is considerably heavier than in the basal medium with one of the

Figs. 55-60. *Rhodopseudomonas gelatinosa.* Magnified × 800
55-57. Four-day-old yeast extract cultures of strains No. 37, 104, and 115 respectively; homogenized with glass beads, incompletely so in Fig. 56.
58-60. Five-day-old homogenized cultures of strain No. 62, in basal medium with Na-formate, acetate, and succinate respectively.

various organic substrates, does the appearance differ from the above; in such cultures the slimy cell masses appear a dirty, faded brown.

The production of a soluble red pigment has been observed with six of the strains.

Physiology. In yeast extract, growth has been obtained when the initial pH of the medium ranged from 6 to 8.5. The temperature relations of none of the strains are accurately known.

The cultures possess an acrid odor, particularly when, as in yeast extract, growth has been appreciable.

On aerobic yeast agar plates many of the strains fail to develop, but none can

be considered a strict anaerobe. All will grow normally when inoculated into a relatively shallow layer of yeast extract, incubated under aerobic conditions.

The most outstanding physiological property of this group of cultures is that they all liquefy gelatin.

Biochemical characteristics. As in the case of *R. spheroides* growth is adversely effected by many of the higher fatty acids; transfers in propionate media do not develop. Apart from the complex substrates like yeast extract or peptone, the best single substrates appear to be ethanol, glucose, fructose, and mannose, and the amino acids alanine, asparagine, aspartic and glutamic acids. The
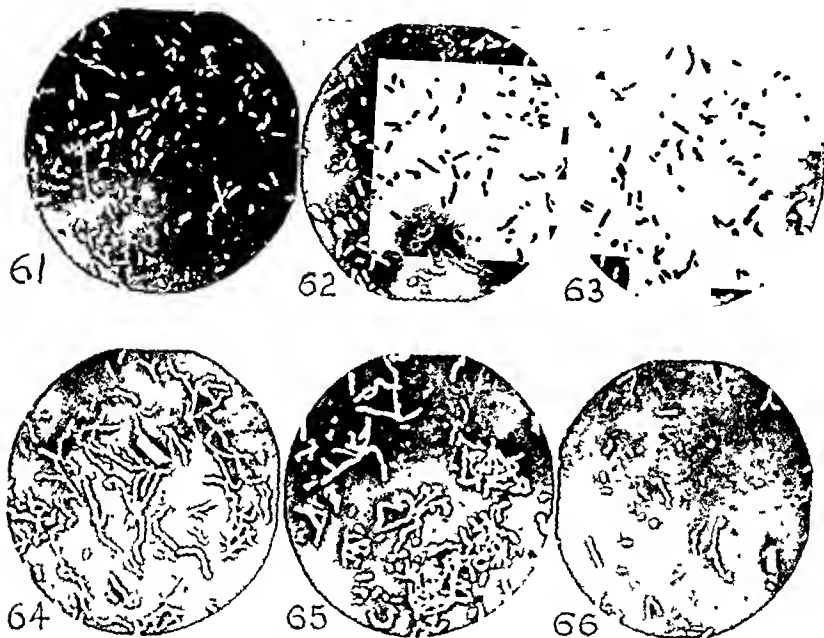
Figs 61–66   *Rhodopseudomonas gelatinosa,* × 800
61–63   Seven-day-old homogenized cultures of strain No 67, in basal medium with Na-acetate, n-butyrate, and lactate respectively.
64–66   Thirty-day-old homogenized cultures of strain No 38, in basal medium with Na-lactate, ethanol, and asparagine respectively

representatives of this group show considerable growth also in citrate. On the other hand, glycerol, mannitol, sorbitol and tartrate do not allow development. Leucine gives rise to poor cultures with about half of the strains; the rest do not grow.

No action on thiosulfate has been detected; whether any of the strains can oxidize molecular hydrogen has not yet been investigated.

Distinguishing characteristics. The combination of morphology, particularly the small size of the individual cells and the stringiness of the cultures, the unusual color of the cell masses, the ability to liquefy gelatin and to utilize amino acids and citrate serve to mark this group off sharply. Significant negative characteristics are the failure to grow in propionate, tartrate, and glycerol.

Enrichment cultures. The most certain way for isolating representatives of this species consists of making enrichment cultures in ethanol, and after some subcultures in the same medium, which serve to reduce the number of colorless organisms introduced with the inoculum, transferring these to citrate, or to a medium with one of the four amino acids which support good growth.

Since streak cultures on aerobic plates often give negative results, pour plates with yeast gelatin, and subculturing from liquefying colonies of purple bacteria, rapidly and certainly lead to the desired result.

Table 12 may serve to summarize the most important characteristics of the various species of the genus *Rhodopseudomonas* here described. The key for the identification of the species of non-sulfur purple bacteria, to be found in the next section. is in part based upon this table.

<div align="center">

Rhodospirillum rubrum (Esmarch) Molisch

Synon.   *Spirillum rubrum* Esmarch

Rhodospirillum photometricum Molisch

Rhodospirillum giganteum Molisch

Rhodospirillum longum Hama

Rhodospirillum gracile Hama

</div>

Twenty strains of non-sulfur purple spirilla have been included in the present study. Sixteen of these are red, the others brown in various culture media. The former are provisionally considered as a single species, *Rhodospirillum rubrum*. One strain was originally obtained from the National Type Culture Collection. Lister Institute. London. and presumably represents a subculture of the original Esmarch strain. All others are new isolations, performed in Delft. Holland. and in Pacific Grove. California   The source material for two of these was an impure culture of *Rhodobacillus palustris*. kindly furnished by Dr E. Schneider, Breslau, Germany. The remaining seventeen strains were isolated from enrichment cultures started with different mud and water samples.

The comparative study of the sixteen red strains has revealed the existence of differences. especially in morphological respect. Some of these differences appear rather marked, even to the extent of suggesting the advisability of a segregation of distinct species (figs. 11–16; 67–96). But the variable morphology of each individual strain (figs. 11–16; 67–75) makes it exceedingly difficult to propose a rational subdivision because the customarily used and easily ascertainable morphological criteria for characterizing spirilla overlap so considerably as to obliterate their usefulness as specific characters. Furthermore, a number of strains, when originally isolated, presented certain features which were apparently quite unlike those of previously studied red spirilla. In some cases this must be ascribed to special culture conditions realized during the isolation procedure; in others it appears clearly the result of gradual modifications in the behavior of particular strains. Noticeable color differences which depend upon the composition of the culture medium must be assigned to the former category; in the latter belong such phenomena as the earlier mentioned changes in the degree of tolerance for oxygen. In the course of time such initially observed differences have, therefore, tended to disappear more or less completely. Thus it became increasingly clear that the distinguishing features did not satisfy the

TABLE 12

*Summary of characteristics of the Rhodopseudomonas species*

Species name	Morphology (Young cultures) Shape	Size in μ	Special Features	Color Anaerobic	Color Aerobic	Slime production	Gelatin liquefaction	Propionate	Glutarate	Tartrate	Citrate	Ethanol	Glycerol	Mannitol	Sorbitol	Glucose	Mannose	Alanine	Leucine	Asparagine	Aspartic acid	Glutamic acid	Thiosulfate
R. palustris	Rods	0.7–0.8 × 1.2–2	Long rods bent, branched ("Mycob.") in older cultures	Red to browned	Red to browned	−	−	+	±	−	−	±	+	−	−	−	−	+	+	•	∓	−	+
R. capsulatus	Cocci at pH <7, rods at pH >7	1 × 2–2.5	Chains of cells in zigzag arrangement	Brown	Red	Above pH 8	−	∓	+	−	−	−	+	−	+	+	+	−	+	−	∓	−	
R. sphæroides	Cocci	0.7 to 1.0	No chains, swollen involution forms	Brown	Red	Above pH 7	−	+	+	−	+	+	+	+	+	+	∓	+	∓	∓	∓	−	
R. gelatinosa	Rods	0.5 × 1–2	Clumped, curved involution forms, thin and long	Pale peach color	−	Very marked	+	∓	−	+	+	−	−	−	+	+	∓	−	∓	+	+	−	

∓ indicates that some strains develop, others fail to grow
∓ indicates that a few strains develop
! indicates exceptionally copious development

requirements of adequate differential characteristics. On the other hand, it has occasionally happened that a strain which had been in the collection for several years was observed to yield, upon plating, colonies of more than one type, especially as far as the morphology of the individual cells was concerned. Observations of this kind have been followed by re-isolation of each one of the
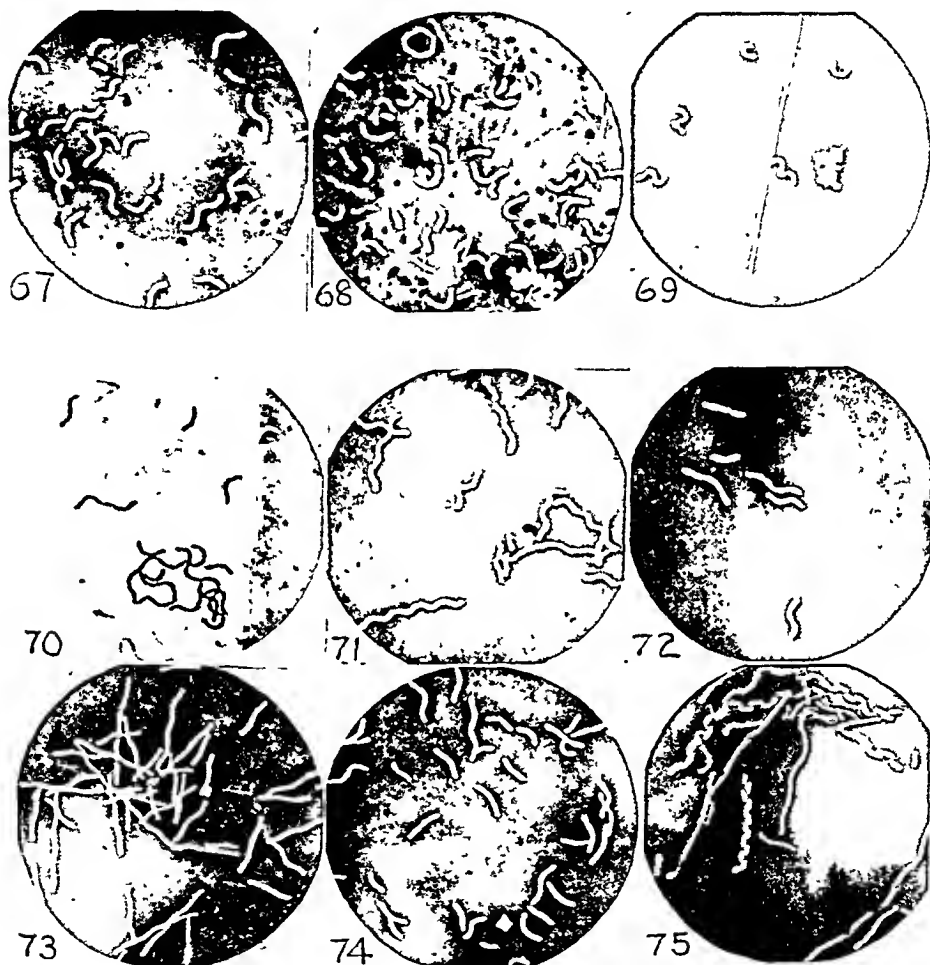
FIGS. 67–75. *Rhodospirillum rubrum*. Strain No. 2. Magnified 800 ×.
67–69. Five-day-old. anaerobic cultures in yeast extract at pH 7.0. 7.5. and 8 0 respectively.
70–75. Seven-day-old. anaerobic cultures in basal medium with Na-*n*-butyrate, succinate. glutarate, malate. ethanol. and asparagine respectively.

distinctive types, and by a careful comparison of the new isolates. The results have been uniform in all cases; the various sub-strains did not exhibit consistent differences either among themselves or with other members of the group.

In view of these facts it seems at present impossible to propose a satisfactory subdivision of the sixteen red strains. It is, of course, possible that future investigations may lead to the discovery of appropriate means for a more refined

classification. As in previous instances, it would appear that attempts of this kind will be less hampered if at this time the common characteristics only are considered.

As a specific name for the group of red spirilla *Rhodospirillum rubrum* (Esmarch) Molisch will be retained. It is hard to see on what basis Molisch proposed two
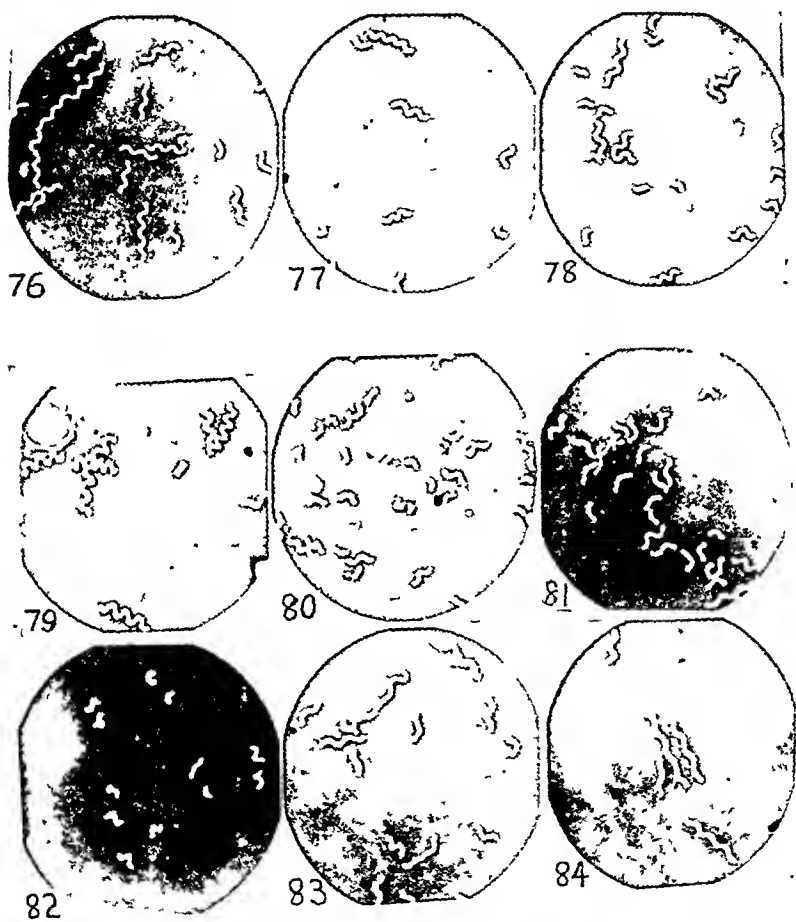
FIGS. 76-84. *Rhodospirillum rubrum*. Strain No 4 × 800
76-78. Three-day-old, anaerobic cultures in yeast extract at pH 6 0. 7 0 and 8 0 respectively.
79-84. Eight-day-old anaerobic cultures in basal medium with Na-*i*-butyrate *n*-caproate, succinate, glutarate, fumarate and malate respectively

new species, *Rhodospirillum photometricum* and *Rhodospirillum giganteum*, in addition to Esmarch's organism which he included in the genus as *Rhodospirillum rubrum* (Esmarch) Molisch (4, p. 24-25). In Esmarch's account the width of this first spirillum to be obtained in pure culture is fixed as "etwa doppelt so stark wie die der Choleraspirillen" (36, p. 227), which would be around 1 μ The

length is emphatically stated as extremely variable; from a gelatin culture cells with 1 to 2 or 3 turns were observed, while in broth at 37°C the individuals were much longer, many measuring 30 to 40, and even up to 50 complete turns. Migula gives more precise data, and lists the width as 1 to 1.2 μ, the length quite indefinite, often reaching 100 μ (129, p. 1027).

From Molisch's statement concerning Esmarch's organism: "Wie ich mich überzeugte, enthält diese Bakterie Bakteriopurpurin und Bakteriochlorin ..." (4, p. 25), one must obviously conclude that he examined an authentic strain. Yet, Molisch neither furnishes morphological data concerning this species, nor does he make clear in what particular respect it differs from *Rhodospirillum photometricum* and *Rhodospirillum giganteum*, of which only the former was studied in pure culture. It is described as 5 to 13 μ long and 1.4 μ thick: the second species, "Sehr nahe verwandt und übereinstimmend," was measured as 9 to 70 μ long by about 1.2 μ thick.

Since pure culture studies have shown that also the width may vary considerably in one and the same strain, I see no reason for maintaining Molisch's species side by side with *Rhodospirillum rubrum*. The same argument applies to Hama's *Rhodospirillum longum* and *Rhodospirillum gracile*, both described from crude cultures only (50,155) and differentiated from previously described species on the basis of cell sizes. The latter are published as 1–1.2 μ wide by 7–250 μ, generally 7–30 μ long, and 1 μ wide by 5–16 μ long respectively.

Morphology. The individual cells are characteristically spiral-shaped. However, the size of the elements is variable within rather wide limits. Depending upon environmental conditions the organisms may be from 0.5 to 1.5 μ wide; in one and the same strain, even in a single culture—with progressive development the composition of the medium may undergo considerable changes!—the dimensions may vary this much. Also the length is far from constant; small spirilla, representing one-half of a complete turn, are usually about 2 to 3 μ long, but elements of 25 to 50 μ in length can frequently be encountered. Nor is the length and width of the turns a fixed morphological character. The following data, pertaining to cultures of one strain and in yeast extract media only, may serve as an illustration.

CELLS		SPIRALS	
Width	Length	Width of turn	Length of turn
0.5–1.2μ	2–50μ	1–4μ	1.5–7μ

In different media the variations appear even more pronounced. At the same time, a more or less consistent relation between the shape and size of the cells and the composition of the medium here becomes apparent. Among the most striking examples of this pattern is the microscopic aspect of cultures in the basal medium with alanine and with malate. In the former, the majority of the cells is found in the form of half-circles to complete rings (Figs. 91–93); in the latter all strains exhibit a tendency towards the formation of elements which are much flattened out in appearance (figs. 73; 88–90).

Among the special involution forms, often consisting of straightened spirals, may be mentioned the irregularly swollen cells characteristic of media with higher fatty acids in initial concentration of over 0.05%. Such organisms stain irregularly, contain fatty inclusions, and are sometimes clearly branched (figs. 94–96).

None of the strains produces mucus. In calcium-deficient media the growth is flocculent, as if agglutinated. It may then form a loose to very compact sediment, or adhere to the sides of the culture vessels. The majority of the individual cells are immotile in such cultures, and cell masses hard to break up. With an adequate supply of calcium the growth in liquid media is suspended and
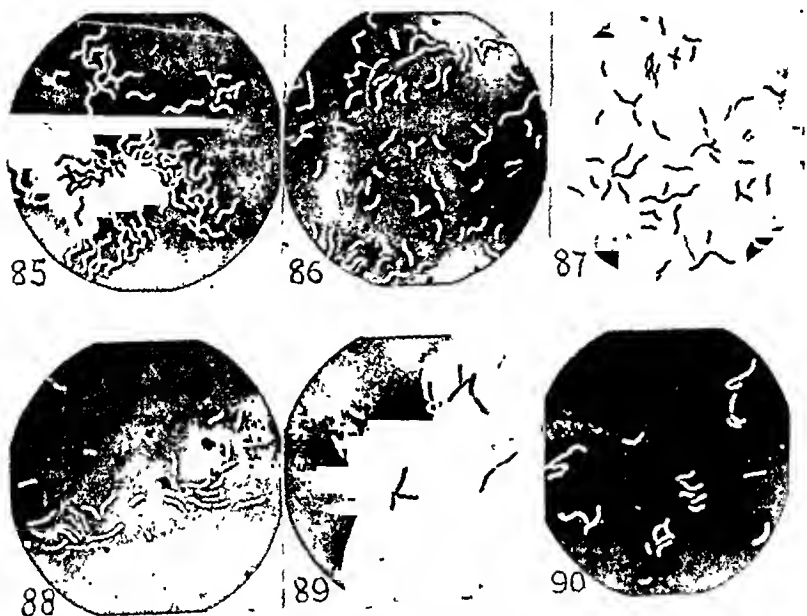
FIGS. 85–90. *Rhodospirillum rubrum.* Magnified × 800.
85–87. Anaerobic cultures in yeast extract at pH 8.0. of strains No. 7, 9, and 10 respectively.
88–90. Anaerobic malate cultures of strains No. 6, 11, and 1 respectively.

consists of separate, motile individuals. The sediment developing in old cultures of this kind can readily be re-dispersed. While anaerobic cultures display an even distribution of the bacteria as long as growth proceeds, a distinct layering becomes apparent upon admission of oxygen. The behavior of individual strains then is that of more or less pronounced micro-aerophils.

Color. Ordinarily deep and dark red, without any brownish tinge. In ethanol media it is of a decidedly different shade, and characteristically pink.

Pigment production is markedly inhibited by the presence of oxygen and the absence of light, the former apparently having an even greater effect than the latter. Thus slants, incubated in the dark, present a pale, greyish surface growth with a very faint reddish hue, while often showing deep-red cell masses

in the region between the glass wall and the agar surface where conditions are still sufficiently aerobic to induce development.

All strains show a marked absorption band with a maximum at about 550 mμ in the intact organisms. None produces a diffusible, water-soluble pigment.

Physiology. Growth in complex media, such as yeast extract, occurs over the entire range tested, *i.e.* from pH 6.0 to 8.5, although it is somewhat retarded in the most acid medium. As with other non-sulfur purple bacteria, the combination of a high fatty acid concentration and a neutral to acid reaction may prevent growth.
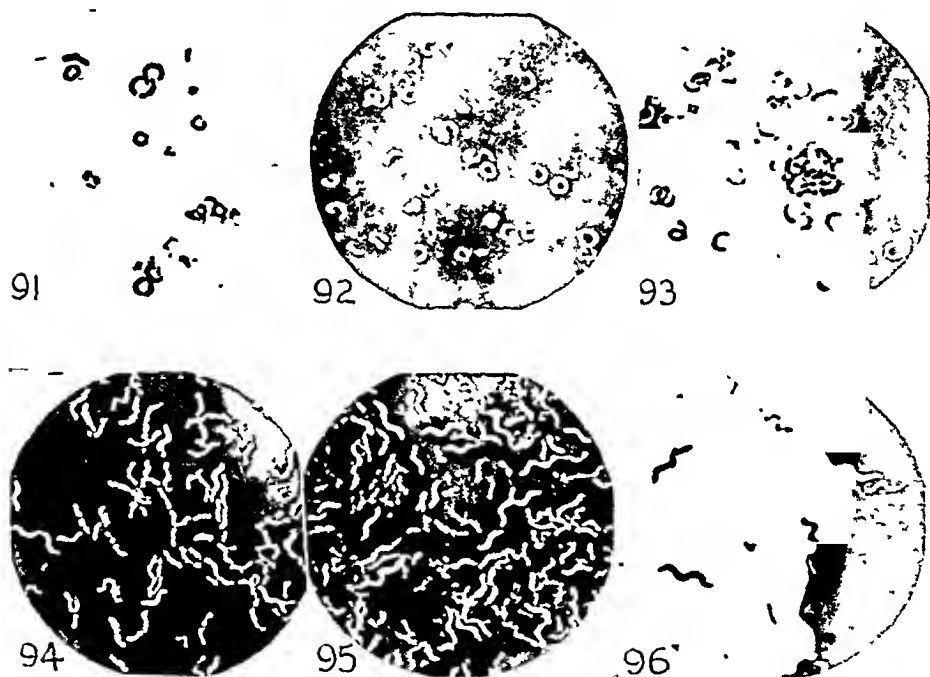
Figs 91–96. *Rhodospirillum rubrum.* × 800
91–93. Anaerobic cultures in basal medium with alanine of strains No. 10, 13 and 15 respectively
94–96. "Involution forms"
94. Strain No. 9 in acetate, 38 days
95. Strain No. 10 in n-butyrate, 26 days
96. Strain No. 11 in glucose, 42 days.

The temperature optimum lies between 30 and 37°C for all strains, and varies slightly with different isolates.

Cultures of *Rhodospirillum rubrum* have a distinctive odor which is, however, difficult to define. It is slightly putrid, and somewhat yeast-like, characteristically different from that of other species of purple bacteria.

Gelatin is not liquefied by any of the strains.

Biochemical characteristics. Growth is generally good with all fatty acids tested, except formate and propionate, in the latter case due to too high a concentration in the experimental media (0.2%). No appreciable development

occurs with tartrate, gluconate, or citrate. Ethanol is a favorable substrate, whereas the carbohydrates and their corresponding polyalcohols are not utilized. Of the amino acids tested alanine, asparagine, aspartic and glutamic acids are satisfactory; glycine and leucine give rise, at best, to slight development.

Not a single strain has been found capable of using thiosulfate. The few isolates which have so far been examined for their ability to oxidize molecular hydrogen have given positive results.

Distinguishing properties. The most important characteristics of the species are the spiral shape combined with the ability to produce a red pigment with a definite absorption maximum at 550 mμ in the intact cells. Diagnostically useful also are the good growth in ethanol, alanine, asparagine, aspartic and glutamic acid media, and the unsuitability of carbohydrates and thiosulfate as substrates.

Enrichment cultures. Most enrichment cultures for non-sulfur purple bacteria contain a larger or smaller proportion of *Rhodospirillum rubrum* cells. Agar shakes from such crude cultures therefore seldom fail to reveal the dark red colonies of this species. More specific enrichment cultures consist of such with various simple alcohols, especially ethanol and *n*-amyl alcohol, or with alanine. By combining its ability to grow in media with both types of substrates it is possible to achieve regularly enrichment cultures in which the majority of organisms consist of *Rhodospirillum rubrum*. In order to forestall an excessive development of *Rhodopseudomonas palustris* it is best to start with alanine media, followed after one or two transfers by cultures in ethanol. The growth of *Rhodopseudomonas capsulatus* is thereby eliminated, while *Rhodopseudomonas gelatinosa* can readily be outgrown by *Rhodospirillum rubrum*, especially if the concentration of yeast autolysate in the enrichment medium does not exceed 0 2%.

Rhodospirillum fulvum

Clearly differentiated from the red *Rhodospirillum rubrum* strains are the brown photosynthetic spirilla. They are here assembled as a single species, *Rhodospirillum fulvum*, readily distinguishable on the basis of a number of morphological and physiological characteristics. Such organisms have not before been described in sufficient detail to make them recognizable, although Molisch and Buder, referring to *Rhodospirillum spec.*, have undoubtedly recorded observations made with representatives of this type. (See pp. 66–68). For this reason a new name has been used here, emphasizing one of the obvious differences from *Rhodospirillum rubrum*.

Unfortunately the four strains of brown spirilla to which I have had access have not been investigated as extensively as the other cultures of non-sulfur purple bacteria. From the beginning they have appeared to be by far the most sensitive members of the group, and could not be cultured except under rigorously anaerobic conditions. In spite of frequent transfers, three strains had perished before the detailed comparative physiological and biochemical studies were started, so that relatively few observations on the morphology under different environmental conditions have been recorded. The one surviving strain with which most of these studies have been carried out has died since.

The following description rests, therefore, mainly on the behavior of a single isolate. Even though the more striking general characteristics have been ascertained for all four strains, this seriously limits its value

Morphology (figs. 97–102). Characteristic for the species is the very small size of the individual cells. According to the available data, they are not over 0.5 μ thick, and rarely longer than 2.5 μ. A single complete turn of about 1 by 1.5 μ represents the most common shape. In media with a higher fatty acid as a substrate the spirals appear steeper than in succinate, fumarate, and malate cultures. But the difference is not great and, in view of the minute size of the cells, difficult to appreciate.
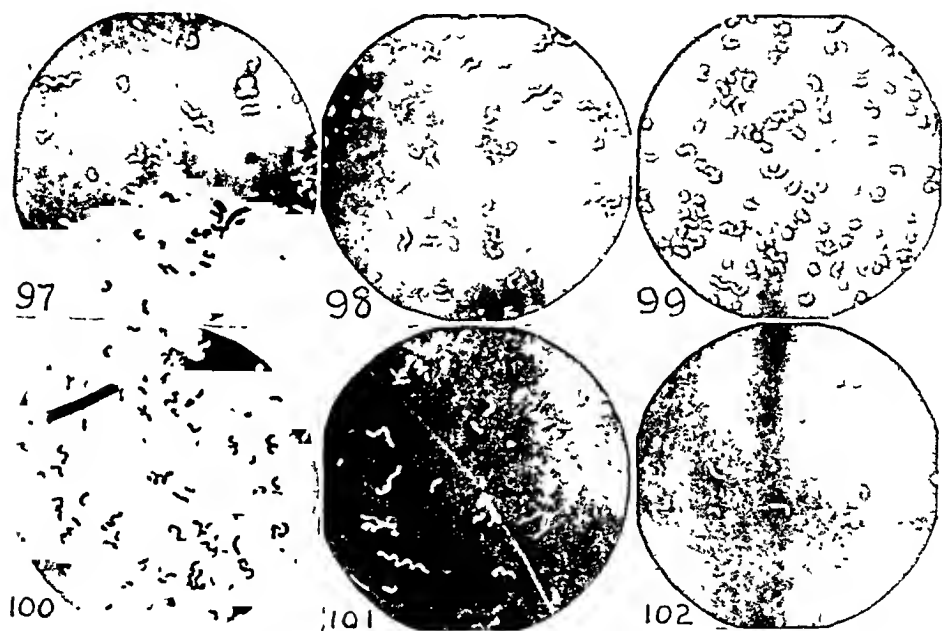
Figs. 97–102. *Rhodospirillum fulvum.* × 800.
Anaerobic cultures of strain No. 123 in basal medium with Na-*n*-butyrate. *n*-caproate, pelargonate, fumarate. malate, and succinate respectively.

Swollen individuals with a resemblance to vibrios can be encountered in cultures which do not appear too healthy. They have been considered as involution forms.

Formation of mucus or clumping in liquid cultures has not been observed.

Color. Quite as distinctive as the size is the color of this species. Individual colonies and stab cultures appear reddish-brown; the shade of certain liquid cultures can be described more accurately as brownish-orange. In agreement herewith is the fact that the absorption maximum at 550 mμ, so characteristic for the red spirilla, is absent in cultures of *Rhodospirillum fulvum.* The production of water-soluble, diffusible pigments has not been observed.

Physiology. No detailed information concerning the effect of the reaction of the medium and of temperature on the development of the brown spirilla is

available; all strains have shown development at 30°C. None has caused gelatin liquefaction.

The behavior towards oxygen indicates that the organism is a strict anaerobe; development in media exposed to air has invariably failed to occur. Even in deep yeast agar columns, rapidly cooled and heavily inoculated by a stab immediately after sterilization, growth is very uncertain if the medium is left without a seal. If a culture is obtained, the upper part, extending over at least 5 cm., remains blank, and subcultures generally fail after more than 10 days.

In agreement with this behavior is the strongly negative acrotaxis, spectacularly evident in liquid media. After lifting the stopper of a uniformly turbid bottle culture, the bacteria disappear rapidly from the upper layers, and in a short period of time the liquid becomes entirely clear with the organisms aggregated at the bottom. Here they may remain actively motile for more than 24 hours provided the culture is not shaken.

Several attempts at adaptation to oxygen have been made, but all with negative results.

Biochemical characteristics. Fatty acids and the four-carbon dicarboxylic acids are uniformly good substrates, while glutaric acid has proved unsuitable for the one strain studied. Abundant development of this isolate has also been observed with ethanol and, curiously, with glucose as a substrate. Other carbohydrates and the corresponding polyalcohols have given negative results. Aspartic acid is the only amino acid which has served as a satisfactory substrate. Thiosulfate is not utilized. No data are available with respect to the oxidation of molecular hydrogen.

Not too much value should be attached to these findings since they are limited to the behavior of a single strain, and, in some cases (glucose, for example) to a single experiment.

Distinguishing properties. The small size of the cells and the color serve as sufficiently characteristic criteria to distinguish this species from the red spirilla. The strictly anaerobic nature and the failure to grow with glutarate and various amino acids except aspartate may be used as supplementary specific properties for this organism.

Enrichment cultures. The present information is entirely inadequate to serve as a guide for selecting specific enrichment media. Successful isolations have been achieved with caprylate and pelargonate media. Whether this is generally satisfactory must, however, await the accumulation of more extensive observations.

IV. Keys for the identification of the species of the non-sulfur purple and brown bacteria

The recognition of only two genera and six species among the non-sulfur purple bacteria makes it a simple matter to devise keys for their determination. The most satisfactory procedure consists of an initial segregation of the two genera, *Rhodopseudomonas* and *Rhodospirillum*, on the basis of cell shape. A rapid determination of the species can then be based upon morphological, physiological or biochemical characters.

Dichotomous keys can be adequately constructed for any one of these various properties. Depending upon the nature of the characters used, one species can usually be singled out immediately. The following keys are examples of some possible arrangements.

Key for the determination of genera:

1. Cells rod-shaped or spherical, not spiral-shaped........... Genus *Rhodopseudomonas*
2. Cells spiral-shaped..... . .. . .Genus *Rhodospirillum*

Key for the determination of Rhosopseudomonas species based upon morphological characters

1. a. Cells clearly rod-shaped in all media 2
   b. Cells more or less spherical in media at pH below 7. 3
2. a. Cells short, somewhat curved, to long branched rods, size 0.7–0.8 by 1.2–2 μ, do not form mucus, liquid cultures evenly turbid. Color red to dark brown-red ...
   .... *Rhodopseudomonas palustris*
   b. Cells slender rods, 0.5 by 1.2 μ, usually clumped together in extensive slime masses. Cultures pale brown to peach-colored . . . . *Rhodopseudomonas gelatinosa*
3. a. In media at pH above 7 clearly rod-shaped, 1 by 1–2.5 μ. Chains of cells frequent in zigzag arrangement . *Rhodopseudomonas capsulatus*
   b. In media at pH above 7 cells still predominantly spherical, 0.7–4 μ in diameter. Mostly single, no chain formation *Rhodopseudomonas spheroides*

Key for the determination of Rhodopseudomonas species, principally based upon physiological properties.

1. a. Gelatin liquefied.... ... . . *Rhodopseudomonas gelatinosa*
   b. Gelatin not liquefied. 2
2. a. Do not produce mucus in media at pH above 8. Color the same under aerobic and anaerobic conditions.................. . . *Rhodopseudomonas palustris*
   b. Produce mucus in media at pH above 8. Color brown under anaerobic, red under aerobic conditions................. 3
3. a. Develop readily in media with 0.2% propionate as the main substrate. Mucus production marked at pH above 8 . ... *Rhodopseudomonas capsulatus*
   b. Do not develop in media with 0.2% propionate as the main substrate. Slime formation evident at pH above 7.0 .... *Rhodopseudomonas spheroides*

Key for determination of Rhodopseudomonas species mainly based upon biochemical properties.

1. a. Thiosulfate used as main oxidation substrate.........*Rhodopseudomonas palustris*
   b. Thiosulfate not used........ 2
2. a. Propionate (0.2%) used.... . ...*Rhodopseudomonas capsulatus*
   b. Propionate not used........ 3
3. a. Mannitol and sorbose utilized. . . ..*Rhodopseudomonas spheroides*
   b. Mannitol and sorbose not utilized . .. .*Rhodopseudomonas gelatinosa*

Key for the determination of Rhodospirillum species.

1. Cultures red; cells over 0.5 μ, usually about 1–1.2 μ wide .*Rhodospirillum rubrum*
2. Cultures brown to orange; cells 0.5 μ or less in width. ....*Rhodospirillum fulvum*

REFERENCES

1. GAFFRON, H. 1933 Über den Stoffwechsel der schwefelfreien Purpurbakterien. Biochem. Z., 260, 1–17.
2. VAN NIEL, C. B. 1929 Photosynthesis of bacteria. In: Contributions to Marine Biology, Stanford Univ. Press, 161–169.
3. VAN NIEL, C. B. 1931 On the morphology and physiology of the purple and green sulphur bacteria. Arch. Mikrobiol., 3, 1–112.
4. MOLISCH, H. 1907 Die Purpurbakterien nach neuen Untersuchungen. G. Fischer, Jena.
5. GAFFRON, H. 1935 Über den Stoffwechsel der Purpurbakterien. II. Biochem. Z., 275, 301–319.
6. FOSTER, J. W. 1940 The rôle of organic substrates in photosynthesis of purple bacteria. J. Gen. Physiol., 24, 123–134.
7. MULLER, F. M. 1933 On the metabolism of the purple sulphur bacteria in organic media. Arch. Mikrobiol., 4, 131–166.
8. VAN NIEL, C. B. 1941 The bacterial photosyntheses and their importance for the general problems of photosynthesis. In: Advances in Enzymology, 1, 263–328.
9. BUDER, J. 1919 Zur Biologie des Bakteriopurpurins und der Purpurbakterien. Jahrb. wiss. Botan., 58, 525–628.
10. WINOGRADSKY, S. 1887 Über Schwefelbakterien. Botan. Ztg., 45, 489 ff, (Nos. 31–37).
11. WINOGRADSKY, S. 1888 Beiträge zur Morphologie und Physiologie der Bakterien. Heft 1; Schwefelbakterien. Arthur Felix, Leipzig.
12. PRINGSHEIM, E. G. 1932 Neues über Purpurbakterien. Naturwissenschaften, 20, 479–483.
13. NADSON, G. A. 1903 Observations sur les bactéries pourprées. Bull. Jard. Imp. Bot. St. Pétersb., 3, 99–109.
14. GAFFRON, H. 1934 Über die Kohlensäure-Assimilation der roten Schwefelbakterien. I. Biochem. Z., 269, 447–453.
15. GAFFRON, H. 1935 Über die Kohlensäureassimilation der roten Schwefelbakterien. II. Biochem. Z., 279, 1–33.
16. VAN NIEL, C. B. 1936 On the metabolism of the *Thiorhodaceae*. Arch. Mikrobiol., 7, 323–358.
17. WINOGRADSKY, S. 1933 Review of (7) in Bull. inst. Pasteur, 31, 972–974.
18. UTERMÖHL, H. 1924 Phaeobakterien. Biol. Zentr., 43, 605–610.
19. EWART, A. J. 1897 On the evolution of oxygen from colored bacteria. J. Linnean Soc. (Bot.), 33, 123–155.
20. CZURDA, V., UND MARESCH, E. 1937 Beiträge zur Kenntnis der Athiorhodobakterien-Gesellschaften. Arch. Mikrobiol., 8, 99–124.
21. LJUBIMENKO, V. 1921 Recherches sur les pigments des bactéries pourpres. J. Soc. Botan. Russie, 6, 107–119.
22. SCHNEIDER, E. 1930 Beiträge zur Physiologie der Farbstoffe der Purpurbakterien. 1. Mitteilung: Die Reinkultur des *Rhodobacillus palustris* Molisch und die Gewinnung seiner Pigmente. Beitr. Biol. Pflanz., 18, 81–115.
23. SELIBER, G. 1928 La culture des bactéries pourprées à la lumière électrique. Bull. inst. Lesshaft, 14, 55–57.
24. SCHRAMMECK, J. 1935 Untersuchungen über die Phototaxis der Purpurbakterien. Beitr. Biol. Pflanz., 22, 314–380.
25. BAARS, J. K. 1930 Over Sulfaatreductie door bacterien. Dissert., Delft.

26. Braak, H. R. 1928 Onderzoekingen over de vergisting van glycerine. Dissert., Groningen.

27. Barker, H. A. 1936 On the fermentation of some dibasic C_4-acids by *Aerobacter aerogenes*. Proc. Koninkl. Akad. Wetenschappen Amsterdam, 39, 674–683.

28. Werkman, C. H., and Wood, H. G. 1942 On the metabolism of bacteria. Botan. Rev., 8, 1–68.

29. Slade, H. D., and Werkman, C. H. 1941 The anaerobic dissimilation of citric acid by cell suspensions of *Streptococcus paracitrovorus*. J. Bact., 41, 675–684.

30. Orla-Jensen, S. 1919 The lactic acid bacteria. Kgl. Danske Videnskb. Selskb. Skrifter, Naturvidenskab math. Afdel., 8, Raekke V, 2.

31. Engelmann, Th. W. 1888 Die Purpurbacterien und ihre Beziehungen zum Licht. Botan. Ztg., 46, 661 ff.; Reprint pp. 1–50.

32. French, C. S. 1937 The rate of CO_2 assimilation by purple bacteria at various wave lengths of light. J. Gen. Physiol., 21, 71–87.

33. French, C. S. 1940 The pigment-protein compound in photosynthetic bacteria. II. The absorption curves of photosynthin from several species of bacteria. J. Gen. Physiol., 23, 483–494.

34. Bavendamm, W. 1924 Die farblosen und roten Schwefelbakterien. G. Fisher, Jena.

35. Forti, A. 1933 Il "Lago di Sangre" a Pergusa in Sicilia e la prima piaga d'Egitto. Il Naturalista Siciliano, Anno 28, Nuova Serie, vol 8°, 1932-XI, 63–86.

36. Esmarch, E. 1887 Ueber die Reincultur eines Spirillum. Centr. Bakt., 1, 225–230.

37. Kluyver, A. J. 1929 Over slijmvorming in melk. Nederland. Tijdschr. Hyg., Microbiol. Serol., 3, 301–307.

38. Gray, P. H. H. 1941 A solution for staining differentially the spores and vegetative cells of micro-organisms. Can. J. Research, 19, 95–98.

39. Lewis, I. M. 1937 Cell inclusions and the life cycle of Azotobacter. J. Bact., 34, 191–204.

40. Lewis, I. M. 1938 Cell inclusions and the life cycle of Rhizobia. J. Bact., 35, 573–586.

41. Lewis, I. M. 1940 The genus *Spirillum* Ehbg. with special reference to cell inclusions and the chromidial theory. J. Bact., 40, 271–284.

42. Lewis, I. M. 1941 The cytology of bacteria. Bact. Rev., 5, 181–230.

43. Hartman, T. L. 1940 The use of Sudan Black B as a bacterial fat stain. Stain Tech., 15, 23–28.

44. Kluyver, A. J., and van Niel, C. B. 1936 Prospects for a natural system of classification of bacteria. Centr. Bakt., II. Abt., 94, 369–403.

45. French, C. S. 1940 The pigment-protein compound in photosynthetic bacteria. I. The extraction and properties of Photosynthin. J. Gen. Physiol., 23, 469–481.

46. Committee on Bacteriologic Technic. Manual of methods for pure culture study of bacteria. Soc. Am. Bacteriologists.

47. Beijerinck, M. W. 1892 La biologie d'une bactérie pigmentaire. Arch. néerland. sci., Haarlem, 25, 227–280. Also in: Verzam. Werken, Delft, 1921, Vol. II, p. 327–358.

48. Giesberger, G. 1936 Beiträge zur Kenntnis der Gattung *Spirillum* Ehbg. Dissert. Utrecht.

49. Myers, J. 1940 Studies on the Spirilleae. J. Bact., 40, 705–720.

50. Hama, Y. 1933 Studien über eine neue Rhodospirillumart aus Yumoto bei Nikko. J. Sci., Hiroshima Univ., Ser. B, Div. 2, 1, 135–156.

51. Beijerinck, M. W. 1902 Photobacteria as a reactive in the investigation of the chlorophyllfunction. Proc. Koninkl. Akad. Wetenschappen., Amsterdam, 4, 5–9.

52. van Niel, C. B. 1935 Photosynthesis of bacteria. Cold Spring Harbor Symposia, 3, 138–150.

53. van Niel, C. B. 1937 Les photosynthèses bactériennes. Bull. assoc. diplomés microbiol. faculté phar. Nancy, Nr. 13, 3–18.

54. Roelofsen, P. A. 1935 On photosynthesis of the Thiorhodaceae. Dissert. Utrecht.

55. CORYELL, C. D. 1940 The proposed terms "exergonic" and "endergonic" for thermodynamics. Science, 92, 380.

56. PREVOT, A.-ROMAIN 1933 Études de systématique bactérienne. I. Lois générales. II. Cocci anaérobies. Ann. sci. nat., Botan., 10ᵉ Série, 15, 23–261.

57. NAKAMURA, H. 1937 Über die Photosynthese bei der schwefelfreien Purpurbakterie, *Rhodobazillus palustris*. Beiträge zur Stoffwechselphysiologie der Purpurbakterien, I. Acta Phytochim., 9, 189–229.

58. NAKAMURA, H. 1937 Über die Kohlensäureassimilation von *Rhodospirillum giganteum*. Beiträge zur Stoffwechselphysiologie der Purpurbakterien, II. Acta Phytochim., 9, 231–234.

59. VAN NIEL, C. B. 1943 Biochemical problems of the chemo-autotrophic bacteria. Physiol. Rev., 23, 338–354.

60. LWOFF, A. 1936 Études sur les fonctions perdues. Ann. fermentations, 2, 419–428.

61. LWOFF, A. 1938 Les facteurs de croissance pour les microorganismes. Ann. inst. Pasteur, 61, 580–617.

62. VAN NIEL, C. B. AND SMITH, J. H. C. 1935 Studies on the pigments of the purple bacteria. Arch. Mikrobiol., 6, 219–229.

63. KLUYVER, A. J. AND CUSTERS, M. TH. J. 1940 The suitability of disaccharides as respiration and assimilation substrates for yeasts which do not ferment these sugars. *Antonie van Leeuwenhoek, 6, 121–162.*

64. STANIER, R. Y. 1942 Are there obligate cellulose-decomposing bacteria? Soil Sci., 53, 479–480.

65. SELIBER, G. 1924 La décomposition des grasses par les bactéries pourprées. Bull. inst. Leshaft, 9, 229–234.

66. PIGULEWSKI, G. UND CHARIK, M. 1928 Zersetzung des Olivenöls unter dem Einfluss der vitalen Tätigkeit einiger Mikroorganismen. Biochem. Z., 200, 201–210.

67. HEILBRUNN, L. V. 1940 Protoplasm and colloids. In: The Cell and Protoplasm, Am. Assoc. Advancement Sci. Publication No. 14, 188–198.

68. ROELOFSEN, P. A. 1934 On the metabolism of the purple sulphur bacteria. Proc. Koninkl. Akad. Wetenschappen, Amsterdam, 37, 660–669.

69. FRENCH, C. S. 1937 The quantum yield of hydrogen and carbon dioxide assimilation in purple bacteria. J. Gen. Physiol., 20, 711–735.

70. WESSLER, S., AND FRENCH, C. S. 1939 The photosynthetic quotient H_2/CO_2 for *Streptococcus varians*. J. Cellular Comp. Physiol., 13, 327–334.

71. NAKAMURA, H. 1937 Über das Vorkommen der Hydrogenlyase in *Rhodobacillus palustris* und über ihre Rolle im Mechanismus der bakteriellen Photosynthese. Acta Phytochim., 10, 211–218.

72. NAKAMURA, H. 1938 Über die Rolle der Hydrogenase im Stoffwechsel von *Rhodobacillus palustris*. Acta Phytochim., 10, 259–270.

73. YAMAGATA, S., UND NAKAMURA, H. 1938 Über die Hydrogenase, nebst einer Bemerkung über den Mechanismus der bakteriellen Knallgasreaktion. Acta Phytochim., 10, 297–311.

74. DEN DOOREN DE JONG, L. E. 1926 Bijdrage tot de kennis van het mineralisatieproces. Dissert. Delft; Nijgh & van Ditmar, Rotterdam.

75. DEN DOOREN DE JONG, L. E. 1927 Über protaminophage Bakterien. Centr. Bakt., II. Abt., 71, 193–232.

76. JANKE, A. 1928 Über den dissimilatorischen Abbau niederer Alkylamine durch Bakterien. Centr. Bakt., II. Abt., 74, 25–26.

77. BARKER, H. A. 1936 The oxidative metabolism of the colorless Alga, *Prototheca zopffii*. J. Cellular Comp. Physiol., 8, 231–250.

78. CLIFTON, C. E. 1937 On the possibility of preventing assimilation in respiring cells. Enzymologia, 4, 246–253.

79. CLIFTON, C. E. AND LOGAN, W. A. 1939 On the relation between assimilation and respiration in suspensions and in cultures of Escherichia coli. J. Bact., 37, 523–540.

80. WINZLER, R. J., AND BAUMBERGER, J. P. 1938 The degradation of energy in the metabolism of yeast cells. J. Cellular Comp. Physiol., 12, 183–211.

81. WINZLER, R. J. 1940 The oxidation of acetate by baker's yeast. J. Cellular Comp. Physiol., 15, 343–354.

82. DOUDOROFF, M. 1940 The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila*. Enzymologia, 9, 59–72.

83. VAN NIEL, C. B. 1940 The biochemistry of microorganisms. In: The Cell and Protoplasm. Am. Assoc. Advancement Sci., Publication No. 14, p. 106–119.

84. BARKER, H. A. 1936 On the biochemistry of the methane fermentation. Arch. Mikrobiol., 7, 404–419.

85. BARKER, H. A., RUBEN, S., AND KAMEN, M. D. 1940 The reduction of radioactive carbon dioxide by methane-producing bacteria. Proc. Natl. Acad. Sci., U.S., 26, 426–430.

86. BARKER, H. A. 1940 Studies upon the methane fermentation. Antonie van Leeuwenhoek, 6, 201–220.

87. BARKER, H. A. 1941 Studies on the methane fermentation. V. Biochemical activities of *Methanobacterium omelianskii*. J. Biol. Chem., 137, 153–167.

88. GLADSTONE, G. P., FILDES, P., AND RICHARDSON, G. M. 1935 Carbon dioxide as an essential factor in the growth of bacteria. Brit. J. Exptl. Path., 16, 335–348.

89. PIRIE, N. W. 1937 The meaninglessness of the terms life and living. In: Perspectives of Biochemistry, Cambridge Univ. Press, p. 11–22.

90. ENGELMANN, TH. W. 1883 *Bacterium photometricum*. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes. Pflügers Arch. ges. Physiol., 30, 95–124.

91. ENGELMANN, TH. W. 1888 Die Purpurbakterien und ihre Beziehungen zum Licht. Bot. Zeitg., 46, 661 ff. Also in Arch. Néerland., 23, 151–198.

92. EWART, A. J. 1897 Bacteria with assimilatory pigments, found in the tropics. Ann. Botany, 11, 486–487.

93. NADSON, G. A. 1903 Observations sur les bactéries pourprées. Bull. Jard. Imp. Bot. St. Pétersb., 3, 109–119.

94. ARCICHOVSKIJ, V. 1904 Zur Frage über das Bakteriopurpurin. Bull. Jard. Imp. Bot. St. Pétersb., 4, 81–98.

95. LÉVY R., TEISSIER, G., ET WURMSER, R. 1925 Étude des pigments d'une bactériacée sulfureuse. Ann. physiol. physicochim. biol., 1, 298–311.

96. VERMEULEN, D., WASSINK, E. C., AND REMAN, G. H. 1937 On the fluorescence of photosynthesizing cells. Enzymologia, 4, 254–268.

97. FRANCK, J., AND GAFFRON, H. 1941 Photosynthesis, Facts and Interpretations. Advances in Enzymology, 1, 199–262.

98. WOHL, K. 1941 On the mechanism of photosynthesis in purple bacteria and green plants. New Phytologist, 40, 34–55.

99. KATZ, E., AND WASSINK, E. C. 1939 Infrared absorption spectra of chlorophyllous pigments in living cells and in extra-cellular states. Enzymologia, 7, 97–112.

100. WASSINK, E. C., KATZ, E., AND DORRESTEIN, R. 1939 Infrared absorption spectra of various strains of purple bacteria. Enzymologia, 7, 113–129.

101. FRENCH, C. S. 1938 The chromoproteins of photosynthetic purple bacteria. Science, 88, 60–62.

102. MESTRE, H. 1930 The investigation of the pigments of the living photosynthetic cell. In: Contrib. to Marine Biology, Stanford Univ. Press, p. 170–187.

103. MESTRE, H. 1935 The absorption of radiation by leaves and algae. Cold Spring Harbor Symp., 3, 191–209.

104. STOLL, A., UND WIEDEMANN, E. 1938 Chlorophyll. In: Fortschr. Chem. organ. Naturst., 1, 159–254.

105. SMITH, E. L. 1940 Chlorophyll as the prosthetic group of a protein in the green leaf. Science, 91, 199–200.

106. Smith, E. L. 1941 The chlorophyll-protein compound of the green leaf. J. Gen. Physiol., 24, 565–582.

107. Smith, E. L. and Pickles, E. G. 1941 The effect of detergents on the chlorophyll-protein compound of spinach as studied in the ultracentrifuge. J. Gen. Physiol., 24, 753–764.

108. Schneider, E. 1934 Über chlorophyllartige Farbstoffe bei den Purpurbakterien. Ber. deut. botan. Ges., 52, 96–100.

109. Schneider, E. 1934 Über das Bakteriochlorophyll der Purpurbakterien. Z. physiol. Chem., 226, 221–254.

110. Fischer, H., Riedmair, J., und Hasenkamp, J. 1934 Über Oxo-porphyrine: Ein Beitrag zur Kenntnis der Feinstruktur von Chlorophyll a. Ann. Chem., 508, 224–249.

111. Fischer, H., und Hasenkamp, J. 1935 Über die Konstitution des Farbstoffes der Purpurbakterien und über 9-Oxy-desoxo-Phäoporphyrin a_5. Ann. Chem., 515, 148–164.

112. Fischer, H., und Lambrecht, R. 1937 Über Bacteriochlorophyll a. Z. physiol. Chem., 249, I–III.

113. Fischer, H., Lambrecht, R., und Mittenzwei, H. 1938 Über Bacterio-Chlorophyll. Z. physiol. Chem., 253, 1–39.

114. Fischer, H., Lautsch, W., und Lin, K. H. 1938 Teilsynthesen von Dehydro-bacterio-phorbid und Dehydro-bacterio-chlorin. Ann. Chem., 534, 1–22.

115. Fischer, H., und Wenderoth, H. 1939 Zur Kenntnis von Chlorophyll. Ann. Chem., 537, 170–177.

116. Fischer, H., Mittenzwei, H., und Hevér, D. B. 1940 Überführung von Dehydro-bacterio-phäophorbid a in Chlorophyll a. Ann. Chem., 545, 154–178.

117. van Niel, C. B., and Arnold, W. 1938 The quantitative estimation of bacterio-chlorophyll. Enzymologia, 5, 244–250.

118. van Niel, C. B. 1933 In: Year Book No. 32 of the Carnegie Institution of Washington, p. 184.

119. Karrer, P., und Solmssen, U. 1935 Die Carotinoide der Purpurbakterien I. Helv. Chim. Acta, 18, 1306–1315.

120. Karrer, P., und Solmssen, U. 1936 Die Carotinoide der Purpurbakterien II. Über Rhodoviolascein. Helv. Chim. Acta, 19, 3–5.

121. Karrer, P., und Solmssen, U. 1936 Carotinoide aus Purpurbakterien III. Helv. Chim. Acta, 19, 1019–1024.

122. Karrer, P., Solmssen, U., und Koenig, H. 1938 Carotinoide aus Purpurbakterien IV. Helv. Chim. Acta, 21, 454–455.

123. Karrer, P., und Koenig, H. 1940 Carotinoide der Purpurbakterien V. Über Rhodoviolascein. Helv. Chim. Acta, 23, 460–463.

124. Schneider, E. 1936 Über die Carotinoide der Purpurbakterien. Beiträge zur Physiologie der Farbstoffe der Purpurbakterien III. Rev. faculté sci. univ. Istanbul, 1, 74–80.

125. French, C. S. 1940 Absorption spectra of the carotenoids in the red and brown forms of a photosynthetic bacterium. Botan. Gaz., 102, 406–409.

126. Mackinney, G. 1940 Plant pigments. In: Ann. Rev. Biochem., 9, 459–490.

127. Migula, W. 1895 Schizophyta. In: Engler und Prantl, Die natürlichen Pflanzenfamilien I, 1a, 1–44.

128. Migula, W. 1897 System der Bakterien, I. Bd., Allgemeiner Teil. G. Fischer, Jena.

129. Migula, W. 1900 System der Bakterien, II. Bd., Specielle Systematik der Bakterien. G. Fischer, Jena.

130. Richter, O. 1907 Die Bedeutung der Reinkultur. Bornträger, Berlin.

131. Jensen, (S.) O. 1909 Die Hauptlinien des natürlichen Bakteriensystems. Centr. Bakt., II. Abt., 22, 305–346.

132. BENECKE, W. 1912 Bau und Leben der Bakterien. Teubner, Leipzig.

133. KRUSE, W. 1910 Allgemeine Mikrobiologie. Vogel, Leipzig.

134. VAHLE, C. 1909 Vergleichende Untersuchungen über die Myxobakteriazeen und Bakteriazeen, sowie die Rhodobakteriazeen und Spirillazeen. Dissert. Marburg. Also in Centr. Bakt., 1910, II. Abt., 25, 178–260.

135. BUCHANAN, R. E. 1917 Studies in the nomenclature and classification of the bacteria. II. The primary subdivisions of the *Schizomycetes*. J. Bact., 2, 155–164.

136. BUCHANAN, R. E. 1918 IX. The subgroups and genera of the *Thiobacteriales*. J. Bact., 3, 461–474.

137. BUCHANAN, R. E. 1925 General systematic bacteriology. Williams & Wilkins, Baltimore.

138. WINSLOW, C.-E. A., BROADHURST, J., BUCHANAN, R. E., KRUMWIEDE, C., ROGERS, L. A., AND SMITH, G. H. 1917 The families and genera of the bacteria. J. Bact., 2, 505–566.

139. BREED, R. S., CONN, H. J., AND BAKER, J. C. 1918 Comments on the evolution and classification of bacteria. J. Bact., 3, 445–459.

140. PRIBRAM, E. 1929 A contribution to the classification of microörganisms. J. Bact., 18, 361–394.

141. NATHANSOHN, A. 1902 Über eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. Mitt. Zool. Stat. Neapel, 15, 655–680.

142. JACOBSEN, H. C. 1912 Die Oxydation von elementarem Schwefel durch Bakterien. Folia Microbiol., 1, 487–496.

143. JACOBSEN, H. C. 1914 Die Oxydation von Schwefelwasserstoff durch Bakterien. Folia Microbiol., 3, 155–162.

144. BEIJERINCK, M. W. 1904 Ueber die Bakterien, welche sich im Dunkeln mit Kohlensäure als Kohlenstoffquelle ernähren können. Centr. Bakt., II. Abt., 11, 593–599. Also in: Verzam. Werken, IV, 242–248.

145. BEIJERINCK, M. W. 1920 Chemosynthesis at denitrification with sulfur as source of energy. Proc. Kon. Akad. Wetensch., Amsterdam, 22, 899–908. Also in: Verzam. Werken, V, 281–288.

146. LIESKE, R. 1912 Untersuchungen über die Physiologie denitrifizierender Schwefelbakterien. Sitzungsber. Heidelb. Akad. Wiss., Abt. B., 1912, 63–91.

147. WAKSMAN, S. A., AND JOFFE, J. S. 1922 Microörganisms concerned in the oxidation of sulfur in the soil. II. *Thiobacillus thiooxidans*, a new sulfur-oxidizing organism isolated from the soil. J. Bact., 7, 239–256.

148. STARKEY, R. L. 1935 Isolation of some bacteria which oxidize thiosulfate. Soil Sci., 39, 197–216.

149. MIYOSHI, M. 1897 Studien über die Schwefelrasenbildung und die Schwefelbacterien der Thermen von Yumoto bei Nikko. J. Coll. Sci., Imp. Univ., Tokyo, 10, 143–173.

150. PRINGSHEIM, E. G. 1923 Zur Kritik der Bakteriensystematik. Lotos, Prag, 71, 357–377.

151. BAVENDAMM, W. 1936 Die Physiologie der schwefelspeichernden und schwefelfreien Purpurbakterien. Ergeb. Biol., 13, 1–53.

152. PRIBRAM, E. 1933 Klassifikation der Schizomyceten. Deuticke, Leipzig.

153. KLUYVER, A. J., AND VAN NIEL, C. B. 1936 Prospects for a natural system of classification of bacteria. Centr. Bakt., II. Abt., 94, 369–403.

154. STANIER, R. Y., AND VAN NIEL, C. B. 1941 The main outlines of bacterial classification. J. Bact., 42, 437–466.

155. HAMA, T. 1933 Nine species belonging to the order *Thiobacteriales* Buchanan, found in Hiroshima. J. Sci. Hiroshima Univ., Ser. B, Div. 2, 1, 157–164.

156. PLOWE, J. Q., SAPERO, J. J., MOY, H. B., AND BECKING, L. B. 1926 A preliminary study of *Rhodobacillus palustris*, Molisch. Proc. Soc. Exptl. Biol. Med., 24, 73–75.

157. UTERMÖHL, H. 1925 Limnologische Phytoplanktonstudien. Arch. Hydrobiol., Suppl. Bd. V, 1–527.

158. RAHN, O. 1929 Contributions to the classification of bacteria. Centr. Bakt., II. Abt., 78, 1–21.

159. ELROD, R. P., AND BRAUN, A. C. 1941 A phytopathogenic bacterium fatal to laboratory animals. Science, 94, 520–521.

160. BELLOC, H. 1910 On Anything. Dutton & Co., New York.

161. PARR, L. W., AND ROBBINS, M. L. 1942 The concept of stability and some of its implications. J. Bact., 43, 661–684.

162. STERN, A., UND PRUCKNER, F. 1940 Lichtabsorption einiger Derivate des Bakteriochlorophylls. Z. physik. Chem., Abt. A, 185, 141–151.

CORRECTION

In the review on The Mode of Action of Sulfonamides, by Richard J. Henry (Bact. Revs., 7, 175–262, 1943) questions were raised with regard to certain experiments by H. I. Kohn and J. S. Harris (J. Pharmacol., 73, 343–361, 1941) because in the original article it was not made entirely clear that the pH was adequately controlled in these experiments. Subsequent data communicated by the authors indicate, however, that the pH was controlled. This removes the basis for the question raised.

EARLY AMERICAN PUBLICATIONS RELATING TO BACTERIOLOGY

I. Textbooks, Monographs, Addresses, Etc.

L. S. McClung[1]

Indiana University, Bloomington, Ind.

(With the assistance of Morris L. Leikind, Library of Congress)

The Committee on Archives of the Society of American Bacteriologists is endeavoring to collect regional histories of the beginnings of bacteriology in America. Several of these have been published as noted by the Chairman of the Committee, Dr. Barnett Cohen[2]. As a possible aid in this program, a compilation has been made of certain publications that have appeared during the early years (through 1915). An immense volume of literature has been released in this period, but only one group of publications is presented in this paper. This, as the title indicates, comprises textbooks, laboratory manuals, monographs, and other longer treatises. Included also are certain compilations, tracts and addresses, which were published independently, or reprinted from the official records of various organizations.

We have omitted purposely, reserving these entries for possible later publication, the original journal articles and the bulletins, circulars or other official publications of the several governmental agencies. For the reason that our records are as yet incomplete, and because they form a separate unit, the list of published doctoral dissertations is omitted for the present. The majority of these appear to occur, at least in abbreviated form, as journal articles. The early reports of the Smithsonian Institution and other organizations, and the Proceedings of the various State Academies of Science are yet to be searched.

It is possible that the future will reveal additional titles, which should have been included in this list, although it is hoped that no serious omissions have occurred[3]. To minimize this, particular attention has been directed to the card catalogues of the several libraries listed below to obtain titles which were later verified. The general list was checked against the Union Catalogue of the Library of Congress. The libraries used in the preparation of this list include the following: Library of Congress; Library of the United States Department of Agriculture; Army Medical Library; New York Public Library; Harvard Medical Library; Boston Medical Library; Yale University, School of Medicine Library; Columbia University, Library of the College of Physicians and Surgeons; Johns Hopkins University, School of Medicine Library; John Crerar Library (including the Union Catalogue of Chicago libraries); the agricultural, general, and medical libraries of the University of Wisconsin; and the general library of Indiana Uni-

versity. The author desires to express his gratitude to the John Simon Guggenheim Memorial Foundation for a fellowship, which permitted the examination of several of these libraries during the course of other studies to which the major portion of the fellowship time was devoted.

The list is divided into two sections. As an aid in conserving space an alphabetical list, by senior author, of all entries is given in Section I. This permits the shortening of the entries for second and later editions of the works cited. This alphabetical listing should appeal also to those who may require this compilation at any time for checking or reference purposes. Although they have been noted whenever found, complete entries have not included reprints of various titles with changes in the date of publication, unless a new edition was announced on the title page or otherwise noted. Without question, additional reprint editions must be in existence, but there is no adequate system for the thorough checking of these since the reprints generally were not copyrighted. A chronological list of authors is given in Section II; the corresponding titles may be obtained easily by reference to Section I.

It has been difficult indeed to determine the limits of subject matter qualifying a work for inclusion in our list. So far as possible, we have interpreted the term "bacteriology" in the strict sense though admitting that it has been difficult at times to decide between bacteriology and one of its sister sciences, pathology, public health, etc. Usually, for example, a monograph relating to pathology is included only if it contains a chapter or a section directly concerned with bacteria or bacteriological techniques. These references, when cited, have been included to show the early relationships between the sciences, but it has not been possible to search out a complete list of this type of material.

We have included translations of foreign publications when the translation was made by an American. It will be noted that such translations have frequently formed the background for a text developed later by the translator. One entry by a Swedish investigator, Arrhenius, is included since it represents a series of lectures delivered at an American university. Occasional references are included to publications by Americans in other countries. All other entries, so far as we have been able to determine, are from authors who were *Americans living and working in this country.*

A small attempt has been made to introduce descriptive notes or comments in the case of certain entries. For the sake of convenience and *uniformity*, use has been made of the notation employed in the Library of Congress Catalogue.

SECTION I

ALPHABETICAL LIST OF AUTHORS AND THE TITLES OF THEIR WORKS

ABBOTT, ALEXANDER CREVER, 1860–1935
 The hygiene of transmissible diseases; their causation, modes of dissemination, and methods of prevention. Philadelphia, W. B. Saunders, 1899. 3, 15–311 p. illus.

———— 2d ed., rev. and enl. with 46 illustrations and 20 charts. 1901. 3 p. l., 15–350 p. illus. (incl. plans) ["Essentially the subject-matter of a portion of my lectures on general hygiene at the University of Pennsylvania."—Pref.]

The principles of bacteriology: a practical manual for students and physicians. Philadelphia, Lea brothers and co., 1892. viii, [13]–263 p. illus.

———— 2d ed., enl. and thoroughly rev. With 94 illustrations, of which 17 are colored. 1894. xi, [13]–471 p. illus. (part col.)

———— 3d ed., enl. and thoroughly rev. With 98 illustrations, of which 17 are colored. 1895. xii, [13]–493 p. illus. (part col.) diagrs.

———— 4th ed., enl. and thoroughly rev ... Philadelphia and New York, Lea brothers and co., 1897. xii, [13]–543 p. illus. (part col.) diagrs.

———— 5th ed., enl. and thoroughly rev. With 109 illustrations, of which 26 are colored. 1899. xi, 17–590 p. illus. (part col.)

———— 6th ed., enl. and thoroughly rev. With 111 illustrations, of which 26 are colored. 1902. xi, 17–641 p. incl. illus. (part col.) diagrs.

———— 7th ed., enl. and thoroughly rev. With 100 illustrations, of which 24 are colored. 1905. xi, 17–689 p. illus. (part col.) diagrs.

———— 8th ed., thoroughly rev. With 100 illustrations, 26 of which are colored. Philadelphia and New York, Lea and Febiger, 1909. xi, 17–631 p. illus. (part col.) col. pl.

———— 9th ed., thoroughly rev., with 113 illustrations, 28 of which are colored. 1915. x, 17–650 p. illus. (part col.) col. plates, diagrs.

ALLEN, RICHARD WILLIAM, 1876–
The bacterial diseases of respiration, and vaccines in their treatment. Philadelphia, P. Blakiston's son and co., 1913. x, 236 p. illus., x pl., fold. diagr. ["Most of the matter ... has already appeared as a series of articles in ... the Journal of Vaccine Therapy from February, 1912, to January, 1913, inclusive. These have been revised and fresh matter included ..."—Pref.]

American public health association.
Disinfection and disinfectants: their application and use in the prevention and treatment of disease, and in public and private sanitation. By the Committee on disinfectants, appointed by the American public health association. Concord, N. H., Republican press association, 1888. 266 p. [First issued in 3 parts as the Report of the Committee on disinfectants, for the years 1885–87, respectively.]

Report of the Committee on disinfectants of the American public health association. 1885– Baltimore, 1885– v. illus.

Public health. The Lomb prize essays. Award made at the thirteenth annual meeting of the American public health association, Washington, D. C., Dec. 10, 1885. With an appendix. 1886. 2 p. l., 198 p. illus. [The essays are also published separately in pamphlet form. *Contents.*—Healthy homes and food for the working classes. By V. C. Vaughan.—The sanitary conditions and necessities of school houses and school life. By D. F. Lincoln.—Disinfection and individual prophylaxis against infectious diseases. By G. M. Sternberg.—Preventable causes of disease, injury, and death in American manufactories and workshops ... By G. H. Ireland.—Appendix.]

Procedures recommended for the study of bacteria, with especial reference to greater uniformity in the description and differentiation of species. Being the report of a com-

mittee of American bacteriologists to the committee on the pollution of water supplies of the American public health association. Submitted at the meeting of the associatian [!] in Philadelphia, Pa., September, 1897. Concord, N. H., The Rumford press, 1898. 47 p. 5 fold. tab.

American public health association. *Laboratory section.*

Standard methods for the bacterial examination of milk and the bacterial examination of air, by committees of the Laboratory section, American public health association. [Boston? 1910?] 48 p. [Reprinted from the American Journal of Public Hygiene, vol. VI, no. 2, May 1910.]

Report of Committee on standard methods of water analysis to the Laboratory section of the American public health association, presented at the Havana meeting, January 9, 1905 ... Chicago, 1905. 1 p. l., 141 p. [Reprinted from the Journal of Infectious Diseases, supplement. no. 1, May 1905.]

... Standard methods for the examination of water and sewage. 2d ed. New York, American public health association, 1912. vi, 144 p. [First edition, 1905, has title: Report of Committee on standard methods of water analysis to the Laboratory section of the American public health association.]

American school of correspondence, *Chicago.*

Bacteriology and sanitation; instruction paper, prepared by Glenn M[oody] Hobbs ... Chicago, Ill., American school of correspondence [°1909] 1 p. l., 77, [4] p. front., illus. [Contains also Examination paper.]

Water supply; a treatise on the sources, distribution, and consumption of water for *commercial and domestic uses, and modern practice in the construction of water works and purification plants*, by Frederick E. Turneaure ... Chicago, American school of correspondence, 1908. 3 p. l., 143 p. front., illus., plates.

American school of home economics, *Chicago.*

Handbook of health and nursing; a complete home-study course, comprising: Household bacteriology, by S. Maria Elliott ... Personal hygiene, by Maurice Le Bosquet ... Home care of the sick, by Amy E. Pope ... Chicago, American school of home economics, 1912. 3 v. in 1. illus., plates. [Each part previously issued separately and contains bibliography.]

... Anatomy, physiology, pathology, bacteriology, dictionary; ed. by W[illiam] A[ugustus] Evans, M.S., M.D., Adolph Gehrmann, M.D., William Healy, A.B., M.D., Chicago, The year book publishers, 1904–06. [The practical medicine series of year books ...]

Anonymous

A manual of biological therapeutics. Press of Parke, Davis and co., 1914. 174 p.

ARCHINARD, PAUL ÉMILE.

... Microscopy and bacteriology. A manual for students and practitioners.... illustrated with seventy-four engravings. Philadelphia and New York, Lea brothers and co. [1903]. 11, 17–210 p. illus., VI col. pl. [The medical epitome series]

... Microscopy, bacteriology, and human parasitology. A manual for students and practitioners. 2d ed., rev. and enl. Illustrated with one hundred engravings and six plates. Philadelphia and New York, Lea and Febiger [°1912]. 2 p. l., 7–267 p. illus., VI col. pl. [The medical epitome series]

ARRHENIUS, SVANTE AUGUST, 1859–1927.

Immunochemistry; the application of the principles of physical chemistry to the study of the biological antibodies. ... New York, The Macmillan Company, 1907. xi, 309 p. incl. tables. diagr. ["The following pages contain a summary of six lectures on the immunity reaction delivered at the University of California, in Berkeley, California, during the summer session of 1904."—Pref.]

AYERS, S. H.   See Biological Studies by the pupils of William Thompson Sedgwick.

BABCOCK, W. WAYNE.   See Shattock, Samuel George.

BALL, MICHAEL VALENTINE, 1868–

. . . Essentials of bacteriology: being a concise and systematic introduction to the study of microorganisms . . . With seventy-seven illustrations, some in colors.   Philadelphia, W. B. Saunders, 1891.   xii, [17]–159 p. incl. front., illus.   [Saunders' question compends, no. 20]

———— 2d ed. With eighty-one illustrations . . . and five plates.   1893.   xiv, [17]–205 p. incl. front., illus. V pl.   [Saunders' question compends, no. 20]

———— 3d ed., rev. With eighty-one illustrations . . . and five plates.   1897. 3 p. 1., xi-xvi, [17]–218 p. front., illus., V pl.   [Saunders' question compends, no. 20]

———— 4th ed., rev.   1900, 236 p. illus.   [Saunders' question compends, no. 20]

———— 5th ed., thoroughly rev. by Karl M. Vogel . . . With ninety-six illustrations, some in colors, and six plates. Philadelphia [etc.] W. B. Saunders and company, 1904. [Also, 1907.] 243 p. incl. front., illus. (part col.) VI pl. [Saunders' question-compends, no. 20]

———— 6th ed., thoroughly rev. With 135 illustrations, some in colors. 1908. 290 p. incl. front., illus. (part col.) tables. [Saunders' question-compends, no. 20]

———— by M. V. Ball . . . assisted by Paul G [arfield] Weston, 7th ed., thoroughly rev. With 118 illustrations, some in colors. 1913. 3 p. 1., 9–321 p. incl. front., illus. (part col.) tables. [Saunders' question-compends, no. 20]

BARKER, LEWELLYS F.   See Langfeld, Millard.

BARNES, CARL LEWIS.

Contagious and infectious diseases; disinfection and disinfectants. An introduction to the subject by Carl L. Barnes . . . [Chicago, The Trade periodical co., 1903] 3 p. 1., 374 p. illus., plates.

BEACH, BENNETT SHELDON.

. . . Histology, pathology, and bacteriology. A manual for students and practitioners. Philadelphia, Lea brothers and co. [c1892] 8, 17–165 p. [Students' quiz series]

BEAM, WILLIAM.   See Leffman, Henry.

BEHRING, EMIL ADOLPH VON, 1854–1917.

The suppression of tuberculosis, together with Observations concerning phthisiogenesis in man and animals and Suggestions concerning the hygiene of cow stables and the production of milk for infant feeding, with special reference to tuberculosis. . . . Authorized translation by Charles Bolduan, M.D. 1st ed. 1st thousand. New York, J. Wiley and sons; London, Chapman and Hall, limited, 1904. V, 85 p.

BELCHER, SARAH DROWNE, "Mrs. E. R. Hardy," 1864–

Clean milk, by S. D. Belcher . . . with an introduction by William Hallock Park . . . New York, The Hardy publishing company, 1903. 146 p. front., 23 pl.

BELFIELD, WILLIAM THOMAS, 1856–

On the relations of micro-organisms to disease. The Cartwright lectures, delivered before the Alumni association of the College of physicians and surgeons, New York, February 19, 21, 24, and 27, 1883. New York, Trow's printing and bookbinding co., 1883. 131 p. illus. [Reprinted from the Medical record. February and March, 1883. Slip pasted over imprint reads: Chicago, W. T. Keener, 1883.]

BERNAYS, AUGUSTUS C.   See Buchanan, Charles Milton.

BEVAN, DAVID.   See Coplin, William Michael Late.

BIGGS, HERMANN MICHAEL, (tr.) See Hueppe, Ferdinand Adolph Theophil.

BIGNAMI, A. See Marchiafava, Ettore.

BIOLETTI, F. T. See Marshall, Charles Edward, (ed.)

Biological studies by the pupils of William Thompson Sedgwick. Published in commemoration of the twenty-fifth anniversary of doctorate. Boston [Printed at the University of Chicago press] 1906. viii, 329 p. front. (port.) 3 pl., diagrs. [Partial Contents: Fuller, G. W. Experimental methods as applied to water- and sewage-works for large communities.—Leighton, M. O. The futility of a sanitary water analysis as a test of potability.—Whipple, G. C. The value of pure water.—Jordan, E. O. Experiments with bacterial enzymes.—Stiles, P. G., and Milliken, C. S. An instance of the apparent antitoxic action of salts.—Winslow, C.-E. A., and Rogers, A. F. A statistical study of generic characters in the Coccaceae.—Prescott, S. C. The occurrence of organisms of sanitary significance on grains.—Gage, S. De M. A study of the numbers of bacteria developing at different temperatures and of the ratios between such numbers with reference to their significance in the interpretation of water analysis.—Winslow, C.-E. A., and Lochridge, E. E. The toxic effect of certain acids upon typhoid and colon bacilli in relation to the degree of their dissociation.—Phelps, E. B. The inhibiting effect of certain organic substances upon the germicidal action of copper sulphate.—Jackson, D. D. A new solution for the presumptive test for Bacillus coli.—Ayers, S. H. B. coli in market oysters.—Wadsworth, Augustus. Studies on simple and differential methods of staining encapsulated pneumococci in smear and section.—Kendall, A. I. An apparatus for testing the value of fumigating agents.—Rickards, B. R. Notes on a case of apparent pulmonary tuberculosis associated with the constant presence of diphtheria-like organisms in the sputum.]

BIRGE, E[DWARD] A[SAHEL].

Synopsis of a course of University extension lectures on bacteriology. Madison, State journal printing co., 15 p., 1892.

BLACK, GREENE VARDIMAN, 1836–1915.

The formation of poisons by micro-organisms. A biological study of the germ theory of disease. Philadelphia, P. Blakiston, son and co., 1884. vi p., 1 l., [11]–178 p.

BOLDUAN, CHARLES FREDERICK, 1873–

Applied bacteriology for nurses, by Charles F. Bolduan . . . and Marie Grund . . . Philadelphia and London, W. B. Saunders company, 1913. 166 p. illus., II col. pl.

_____

Immune sera; a concise exposition of our present knowledge concerning the constitution and mode of action of antitoxins, agglutinins, haemolysins, bacteriolysins, precipitins, cytotoxins, and opsonins . . . 2d ed., rewritten. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1907. viii, 154 p. illus. ["This book has its origin in a monograph by Professor Wassermann, a translation of which was published by the author in 1904 under the title 'Immune sera'. While much of the material contained in that book will be found in the present volume, it has been deemed necessary to discuss more fully the original topics, and to widen the scope of the book by adding chapters. . . "—Pref.]

_____

_____ 3d ed., enl. 1st thousand. 1908. viii, 176 p. illus.

_____

Immune sera; a concise exposition of the main facts and theories of infection and immunity . . . 4th ed., rewritten and enl. 1st thousand. 1911. xi, 226 p. illus.

BOLDUAN, CHARLES FREDERICK. See Behring, Emil Adolph von.

_____ See Dieudonné, Adolf.

_____ See Ehrlich, Paul.

_____ See Rostocki, Otto.

_____ See Wassermann, August von.

BOLDUAN, CHARLES F. See Nuttall, G. H. F.

BOLTON, B. MEADE. See Williams, Herbert Upham.

BORDET, JULES.

Studies in immunity by Professor Jules Bordet . . . and his collaborators; collected and tr. by Frederick P. Gay . . . including a chapter written expressly for this publication by Professor Bordet. 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1909. vii, 545 p. front. (port.)

BOWHILL, THOMAS.

Manual of bacteriological technique and special bacteriology . . . with one hundred original illustrations. New York, W. Wood and company, 1899. xii, 284 p. illus., plates.

BROCA, A. See Senn, Nicholas.

BROOKS, HENRY T. See Lenhartz, Hermann.

BUCHANAN, CHARLES MILTON, 1868–1920.

Antisepsis and antiseptics. With an introduction by Professor Augustus C. Bernays. Newark, N. J., Terhune co., 1895. xvi, [3]–352 p. front. (5 port.) plates.

BUCHANAN, Mrs. ESTELLE DENIS (FOGEL) 1876–

Household bacteriology for students in domestic science, by Estelle D. Buchanan . . . and Robert Earle Buchanan . . . New York, The Macmillan company, 1913. [Also 1914] xv, 536 p. illus.

BUCHANAN, JOHN, M.D.

An encyclopedia of the practice of medicine, based on bacteriology. 1st ed. New York, R. R. Russell, 1890. 1 p. l., 11–1453 p. illus.

BUCHANAN, ROBERT EARLE, 1883–

Veterinary bacteriology; a treatise on the bacteria, yeasts, molds, and protozoa pathogenic for domestic animals . . . with 214 illustrations. Philadelphia and London, W. B. Saunders company, 1911. 1 p. l., 9–516 p. illus. ["A revision of the lectures on veterinary bacteriology given during the past six years to classes in the Division of veterinary medicine in the Iowa state college."]

BUCHANAN, ROBERT EARLE. See Buchanan, Mrs. Estelle Denis.

——— See Marshall, Charles Edward, ed.

BURNHAM, FREDERICK W. E.

Haemocytes and haemic infections; a hand-book for students and practitioners, with two hundred and twenty-six microphotograms by the author. 1st ed. Winnipeg, Manitoba [Akron, O., The New Werner company] 1913. 462 p. incl. illus., plates.

CALDWELL, CHARLES, 1772–1853.

An address to the Philadelphia medial society, on the analogies between yellow fever and true plague, delivered, by appointment, on the 20th of February, 1801. Philadelphia, Printed by Thomas and William Bradford, booksellers and stationers, no. 8, South Front street. 1801. viii, 44 p.

A semi-annual oration, on the origin of pestilential diseases, delivered before the Academy of medicine of Philadelphia, on the 17th day of December, 1798. Philadelphia, Printed by T. and S. F. Bradford, 1799. xii, [13]–59 p.

CAREY, HARRY WARDELL, 1875–

An introduction to bacteriology for nurses . . . Philadelphia, F. A. Davis company; [etc., etc.] 1915. vii, 144 p. illus.

CARTER, WILLIAM S. (comp.)

Notes on the lectures of Prof. John Guitéras on general and special pathology, delivered before the second and third year students of the University of Pennsylvania; and on the lectures of Dr. Joseph McFarland on bacteriology, delivered before the third year class. Arranged by Dr. William S. Carter and Dr. David Riesman . . . [Philadelphia, Avil printing co., 1895] 246, 56 p. illus.

CASHIN, JOHN E. See Pfingst, Adolph O.

CHADWICK, FRENCH ENSOR, 1844–

Temperament, disease and health. New York, [etc.] G. P. Putnam's sons, 1892. vi, 85 p.

CHAPIN, CHARLES VALUE, 1856–

    Municipal sanitation in the United States. illus. Providence, Rhode Island, Snow and Farnham, 1901. viii, 970 p.

―――― The sources and modes of infection . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1910. ix, 399 p.

―――― 2d ed., rev. and enl., first thousand. 1912. xi, 481 p.

CHAPIN, HENRY DWIGHT, 1857–

    Theory and practice of infant feeding, with notes on development . . . 1st ed. New York, W. Wood and company, 1902. ix, 1 l., 326 p.

―――― 2d ed., rev. . . . New York, W. Wood and company, 1904. xi p., 1 l., 342 p. illus. [Chapter XV, "Bacteriological examination of milk," was prepared by H. W. Conn.]

CHESTER, FREDERICK DIXON, 1861–

    A manual of determinative bacteriology, by Frederick D. Chester . . . New York, The Macmillan company; London, Macmillan and co., ltd., 1901. vi p., 1 l., 401 p. illus.

Chicago. University.

    . . . Investigations representing the departments; zoölogy, anatomy, physiology, neurology, botany, pathology, bacteriology . . . Chicago, The University of Chicago press, 1903. x, 396 p. illus., xxxix pl. (part col.) [Chicago. University. The decennial publications. First series, vol. x *Partial Contents.*—The self-purification of streams, by E. O. Jordan.]

CHRYSTIE, WALTER. See Hare, Hobart Amory.

CITRON, JULIUS BERNHARD.

    Immunity; methods of diagnosis and therapy and their practical application . . . tr. from the German and ed. by A. L. Garbat . . . 27 illustrations, 2 colored plates and 8 charts. Philadelphia, P. Blakiston's son and co., 1912. xiii, 209 p. illus. (incl. charts) 2 col. pl.

―――― . . . tr. from the German and ed. by A. L. Garbat . . . 2d ed., rev. and enl.; 30 illustrations, 2 colored plates and 8 charts. Philadelphia, P. Blakiston's son and co. [c1914] xvii, 267 p. illus (incl. charts) 2 col. pl., tables.

COHN, FERDINAND JULIUS, 1828–1898.

    Bacteria: the smallest of living organisms. Tr. by Charles S. Dolley . . . Rochester, N. Y. [Press of F. D. Phinney] 1881. 30 p. pl.

    . . . Über bakterien, die kleinsten lebenden wesen, von dr. Ferdinand Cohn . . . with notes for American students by Oswald Seidensticker . . . New York, H. Holt and company [etc.]; Boston, C. Schoenhof [1889] iii p., 21, 47 p. illus. [German scientific monographs ed. for American students. (no. 2)]

COLE, MARTIN J. See Cross, M. I.

CONDICT, LEWIS, 1773–1862.

    An inaugural dissertation on the effects of contagion upon the human body. Being an attempt to ascertain its mode of *operation,* with a few observations on the proper method of preventing and curing febrile contagious diseases. Submitted to the examination of the Rev. John Ewing, S. T. P., provost, the medical professors and trustees of the University of Pennsylvania, for the degree of doctor of medicine, on the 19th day of May 1794. By Lewis Condict, of New Jersey . . . Philadelphia: Printed by William W. Woodward, at Franklin's head, no. 41, Chestnut-street. 1794. 26 p.

CONN, HERBERT WILLIAM, 1859–1917.

    Agricultural bacteriology; a study of the relation of bacteria to agriculture, with special reference to the bacteria in the soil, in water, in the *dairy,* in miscellaneous farm

products, and in plants and domestic animals . . . Philadelphia, W. Blakiston's son and co., 1901. vii, 17–412 p. illus.

Agricultural bacteriology; a study of the relation of germ life to the farm, with laboratory experiments for students . . . 2d ed., rev. and enl., with 64 illustrations. Philadelphia, P. Blakiston's son and co., 1909. x, 331 p. illus.

Bacteria, yeasts, and molds in the home . . . Boston and London, Ginn and company, 1903. vi, 293 p. illus., diagrs.

———— Rev. ed. Boston, New York etc. Ginn and company [c1912] vi, 295 p. illus.

Bacteria in milk and its products, designed for the use of students in dairying and for all others concerned in the handling of milk, butter or cheese . . . 43 illustrations. Philadelphia, P. Blakiston's son and co., 1903. vii, 17–306 p. illus.

Germ life, bacteria; with thirty-four illustrations. London, Hodder and Stoughton, 1909. 212 p. illus. [Useful knowledge series. First published in 1897. See: The story of germ life]

The living world: whence it came and whither it is drifting; a review of the speculations concerning the origin and significance of life and of the facts known in regard to its development, with suggestions as to the direction in which the development is now tending . . . New York. London, G. P. Putnam's sons, 1891. v p., 1 l., 195 p. illus., diagrs.

. . . Nociones de microbiología, aplicada á la agricultura, la industria, la medicina, etc.; traducida del inglés al español por el Dr. Antonio Soler . . . Nueva York, D. Appleton y cía, 1902. 189 p. illus. [Nuevas cartillas científicas]

Practical dairy bacteriology, prepared for the use of students, dairymen, and all interested in the problems of the relation of milk to public health . . . New York, Orange Judd company; [etc., etc.] 1907. [Also 1914] xi, 1, 314 p. incl. front., illus.

The story of germ life . . . New York, D. Appleton and company, 1897. 199 p. illus. [Half-title: The Library of useful stories] [Also (New York Public Library), 1898, New York; 1899, London, George Newnes, 212 p. illus.; 1900, New York.]

———— 1902. 199 p. illus. [First published in 1897.]

———— New York, S. S. McClure Co., 1909. 199 p. illus. [Library of valuable knowledge]

———— New York, D. Appleton and Co., 1912. 199 p. illus. [Library of valuable knowledge]

———— New York, 1915. 199 p. front., illus. [Lettered on cover: Library of valuable knowledge]

CONN, H[ERBERT] W[ILLIAM]. See Chapin, Henry Dwight.
COPLIN, WILLIAM MICHAEL LATE, 1864–
    Lectures on Pathology; delivered to the students at Jefferson medical college, Philadelphia. Philadelphia, P. Blakiston, son and co. 1894. v, illus., pl. [1st ed. of Manual of Pathology]

———— Manual of pathology, including bacteriology, the technic of postmortems, and methods of pathologic research ... being a 2d ed. of the author's "Lectures on pathology," rewritten and enl.; with two hundred and sixty-eight illustrations ... Philadelphia, P. Blakiston, son and co., 1897. xxi, 11–638 p. illus.

———— 3d ed., rev. and enl., with three hundred and thirty illustrations and seven colored plates ... Philadelphia, P. Blakiston's son and co., 1900. xx, 11–846 p. illus., col. plates.

———— 4th ed., rewritten and enl. With four hundred and ninety-five illustrations, many of which are original, and ten colored plates. Philadelphia, P. Blakiston's son and co., 1905. xxvii p., 1 l., 994 p. illus., X col. pl.

———— 4th ed., rewritten and enl., reprinted with corrections, with four hundred and ninety-five illustrations and twenty-three plates, ten of which are in colors. Philadelphia, P. Blakiston's son and co., 1910. xxvii p., 1 l., 994 p. illus. (partly col.) XXIII pl. (partly col.)

———— 5th ed., *rewritten and enl., with six hundred and twelve illustrations and twelve plates, eleven of which are in colors.* 1911. xxiv, 1139 p. illus. (part col.) XII pl. (11 col.)

———— A manual of practical hygiene ... By W. M. L. Coplin ... and D. Bevan ... With an introduction by H. A. Hare ... 1893. xvi, [25]–456 p. illus., diagrs.

COUNCILMAN, WILLIAM THOMAS, 1854–1933.
Disease and its causes, by W. T. Councilman ... New York, H. Holt and company; [etc., etc., ^c1913] 2 p. l., [iii]–viii, 9–254 p. illus., fold. pl. [*Half-title:* Home university library of modern knowledge. no. 68]

———— Pathology, a manual for teachers and students. Boston, Wm. Leonard, 1912. 405 p.

———— Pathology; syllabus, by W. T. Councilman, M.D., and F. B. Mallory, M.D. ... Boston, Printed by J. L. Fairbanks and company, 1902. iv, 174 p.

CROSS, M. I.
Modern microscopy: a handbook for beginners and students, combining I. The microscope, and instructions for its use, by M. I. Cross; II. Microscopic objects: how prepared and mounted, by Martin J. Cole ... 3d ed., entirely rev. and enl., to which is added III. Microtomes: their choice and use. Chicago, W. T. Keener and co., 1903. xvi, 292 p. incl. front., illus., diagrs. [Printed in Great Britain]

CUTTER, EPHRAIM, 1832–1917.
Partial report on the production of vaccine virus in the United States ... Philadelphia, Collins, printer, 1872. 39 p. illus. ["Extracted from the Transactions of the American medical association."]

DALTON, JOHN C[ALL] 1825–1889.
The origin and propagation of disease. An anniversary discourse, delivered before the New York academy of medicine, November 20, 1873 ... New York, D. Appleton and company, 1874. 30 p.

DEASON, J.
Practical bacteriology, by J. Deason ... Kirksville, Mo., Journal printing company, 1910. 50 p. forms.

———— 2d ed., rev. and enl., 1911. 130 p.

DELAFIELD, FRANCIS, 1841–1915.

A handbook of pathological anatomy and histology, with an introductory section on post-mortem examinations and the methods of preserving and examining diseased tissues, by Francis Delafield . . . and T. Mitchell Prudden . . . 3d ed., illustrated by 224 wood engravings printed in black and colors. New York, W. Wood and company, 1889. xv, 609 p. illus. (part col.)

———— 4th ed.. illustrated by 300 wood engravings printed in black and colors. New York, W. Wood and company, 1892. xvii, 715 p. illus. (part col.)

———— 5th ed., . . . 1896. xviii, 846 p. illus. (part col.) col. pl.

———— 6th ed., . . . 1901. xix, 819 p. illus., XIII pl. (part col.)

———— 7th ed., with 13 full-page plates and 545 illustrations in the text in black and colors. 1904. xxi, 885 p. illus. (part col.) XIII pl. (3 col.)

A text-book of pathology, with an introductory section on post-mortem examinations and the methods of preserving and examining diseased tissues, by Francis Delafield . . . and T. Mitchell Prudden . . . 8th ed., with thirteen full-page plates and six hundred and fifty illustrations in the text, in black and colors. New York, W. Wood and company, 1907. xxv, 1057 p. illus. (part col.) XIII pl. (3 col.)

A text-book of pathology, with a final section on post-mortem examinations and the methods of preserving and examining diseased tissues, by Francis Delafield . . . and T. Mitchell Prudden . . . 9th ed., with thirteen full-page plates and six hundred and eighty-seven illustrations in the text, in black and colors. New York, W. Wood and company, 1911. xxvi, 1114 p. illus. (part col.) XIII pl. (3 col.)

———— 10th ed., rev. with the coöperation of Francis Carter Wood . . . with fourteen full-page plates and six hundred and ninety-four illustrations in the text, in black and colors. New York, W. Wood and company, 1914. xxviii, 1116 p. illus. (part col.) plates (part col.)

DIBBLE, FREDERICK L.

Vagaries of sanitary science. Philadelphia, J. B. Lippincott company, 1893. 462 p.

DICK, GEORGE FREDERICK. See Ricketts, Howard Taylor.

DIEUDONNÉ, ADOLF, 1864–

Bacterial food poisoning; a concise exposition of the etiology, bacteriology, pathology, symptomatology, prophylaxis, and treatment of so-called ptomaine poisoning, by Prof. Dr. A. Dieudonné, Munich; tr. and ed., with additions, by Dr. Charles Frederick Bolduan . . . Authorized translation. New York, E. B. Treat and company, 1909. 3 p. l., 9–128 p.

DOANE, RENNIE WILBUR, 1871–

. . . Insects and disease; a popular account of the way in which insects may spread or cause some of our common diseases, with many original illustrations from photographs . . . New York, H. Holt and company, 1910. xiv, 227 p. front., plates. [American nature series. Group IV. Working with nature.]

DOLLEY, CHARLES S. M.D.

Notes on the methods employed in Biological Studies. Compiled solely for the use of students in the laboratories of the School of Biology, University of Pennsylvania. Published by the University, 1889.

The technology of bacteria investigation; explicit directions for the study of bacteria: their culture, staining, mounting, etc. . . . Boston, S. E. Cassino and company, 1885. xii, 263 p.

DOLLEY, CHARLES S[UMNER]. See Cohn, Ferdinand Julius.

DOTT, ALVAH HUNT, 1854–

Prevention of infectious diseases . . . New York and London, D. Appleton and company, 1911. 5 p. l., 280 p., 1 l.

DUCKWALL, EDWARD WILEY.

Bacteriology. Applied to the canning and preserving of food products. [Baltimore, Md., The Trade, 1899] x p., 1 l., 111 p. illus., plates (part col.)

———

Canning and preserving of food products with bacteriological technique; a practical and scientific hand book for manufacturers of food products, bacteriologists, chemists and students of food problems. Also for processors and managers of food product manufactories . . . 1st ed. Pittsburgh, Pa., Pittsburgh printing company, 1905. 478 p. incl. front. (port.) illus., plates.

EARP-THOMAS farmogerm co., *Bloomfield, N. J.*

Farmogerm, high-bred nitrogen-gathering bacteria; makes poor soil good soil. Bloomfield, N. J., Earp-Thomas farmogerm co. [ᶜ1909]. cover-title, 32 p. illus.

EHRLICH, PAUL, 1854–1915.

Collected studies on immunity, by Professor Paul Ehrlich, privy councilor and director of the Royal institute for experimental therapy, Frankfurt, Germany, and by his collaborators; with several new contributions, including a chapter written expressly for this edition by Professor Ehrlich; tr. by Dr. Charles Bolduan . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1906. 2 p. l., iii–xi, 586 p. front. (port.) illus., diagrs.

———

Studies in immunity, by Professor Paul Ehrlich . . . and his collaborators, collected and tr. by Dr. Charles Bolduan . . . 2d ed., rev. and enl. 1910. 2 p. l., iii–xi, 712 p. incl. illus., tables. front. (port.)

EIMER AND AMEND

Revised and enlarged catalogue of bacteriological apparatus; manufactured or imported by Eimer and Amend. New York, 1907. 222 p. illus.

EISENBERG, JAMES, 1861–

Bacteriological diagnosis: tabular aids for use in practical work . . . Tr. and augm. . . . from the 2d German ed., by Norval H. Pierce . . . Philadelphia and London, The F. A. Davis co., 1892. xiv, 184 p.

ELIOT, CHARLES WILLIAM, 1834–

The fruits of medical research with the aid of anaesthesia and asepticism [by] Charles W. Eliot . . . [Boston, The Barta Press] 1909. 19 p. ["Address delivered at the Massachusetts general hospital on the sixty-third anniversary of Ether day, October 16, 1909."]

ELLIOTT, SOPHRONIA MARIA, 1854–

Household bacteriology . . . [Textbook ed.] Chicago, American school of home economies, 1910. 3 p. l., 170 (i.e. 180 p) p. front., illus., plates, port.

[ERNST, HAROLD CLARENCE] 1856–1922.

Infectiousness of milk; result of investigations made for the trustees of the Massachusetts society for promoting agriculture. Boston, The Society, 1895. 2 p. l., 141 p. incl. tables. xvii pl.

ERNST, HAROLD CLARENCE, 1856–1922.

Modern theories of bacterial immunity by Harold C. Ernst, M.D. Boston, The Journal of medical research, 1903. 3 p. l., 123 p. ["Abstract of a short series of lectures delivered . . . in the Harvard medical school in January, 1903."—Introd.]

———

Progress of medicine. 1850–1900. An oration delivered before the Norfolk District Medical Society at the celebration of the semi-centennial of its organization, May 8, 1900. Boston, Mass. 20 p.

ESHNER, AUGUSTUS ADOLPH, (*tr.*) See Levy, Ernst.
EVANS, W[ILLIAM] A[UGUSTUS]. See . . . Anatomy, physiology, pathology, bacteriology, dictionary, etc., etc.
FAUGHT, FRANCIS ASHLEY, 1881–
    Essentials of laboratory diagnosis, designed for students and practitioners . . . containing an indican scale in colors, six full-page plates and numerous engravings in the text. Philadelphia, F. A. Davis company, 1909. viii, 309 p. front., illus., VI pl. (1 col.)

    ———— containing an indican scale in colors; eight full-page plates and numerous engravings in the text. 2d rev. ed. 1910. xiv, 336 p. front., illus., plates (1 col.)

    ———— containing eleven full-page plates (three in colors) and thirty-nine text engravings. 3d rev. ed. 1911. xii, 338 p. illus., 11 pl. (incl. front., 3 col.)

    ———— 4th rev. ed. 1912. xii, 338 p. illus., 11 pl. (incl. front., 3 col.)

    ———— containing ten full-page plates (four in colors) and fifty-eight text engravings. 5th rev. ed. 1915. xii, 450 p. illus., 10 pl. (4 col.)
FELLOWS, JAMES I.
    Zymosis and pathogenesis; a bacteriological sketch (2d section). New York, n. p., 1892. 128 p. [For the medical profession. pt. XI.]
FITCH, CLIFFORD PENNY. See Moore, Veranus Alva.
FLEXNER, SIMON AND HOLT, L. EMMET (*ed.*)
    Bacteriological and clinical studies of the diarrheal diseases of infancy, with reference to the *Bacillus dysenteriae* (Shiga) from the Rockefeller Institute for Medical Research. New York, Rooney and Otten ptg. co., 1904. 202 p.

    The biological basis of specific therapy by Dr. Simon Flexner . . . [Boston, The Barta press] 1911. 20 p. ["Address delivered at the Massachusetts general hospital on the sixty-fifth anniversary of Ether day, Oct. 16, 1911."]
FLEXNER, SIMON. See Marchiafava, Ettore.
FOX, HERBERT, 1880–
    Elementary bacteriology and protozoölogy, the microbiological causes of the infectious diseases . . . Illustrated with 67 engravings and 5 colored plates. Philadelphia and New York, Lea and Febiger, 1912. vi, [17]–237 p. illus., V col. pl. incl. front.
FRAENKEL, CARL, 1861–
    Text-book of bacteriology, by Carl Fraenkel . . . 3d ed. Tr. and ed. by J. H. Linsley . . . New York, W. Wood and company, 1891. 3 p. l., 376 p.
FRIEDLAENDER, CARL, 1847–1887.
    A manual of microscopical technology for use in the investigations of medicine and pathological anatomy . . . Tr. . . . from the 2d enl. and cor. ed. by Stephen Yates Howell. New York and London, G. P. Putnam's sons, 1885. vi p., 31., 249 p. pl.
FROST, WILLIAM DODGE, 1867–
    Bacteriological tests of methods of cleaning. [*In* National education association of the United States. Journal of proceedings and addresses, 1911. p. 985–990.]

    The great white plague. Simple lessions on causes and prevention. Intended especially for use in schools. By W. D. Frost . . . and M. V. O'Shea . . . Issued by C. P. Cary, state superintendent. Madison, Democrat printing company, state printer, 1912. 48 p. front., illus. (incl. ports.)

    A laboratory guide in elementary bacteriology. Madison, Wis., the author, 1901. 205 p.

———————
————— 2d rev. ed. 1902. x, 355 p. illus., II pl. on 1 l., diagrs.

————— 3d ed., 1903. 395 p.

————— 4th ed. rev., 1911. 395 p.

A text-book of general bacteriology, by William Dodge Frost . . . and Eugene Franklin McCampbell . . . New York, The Macmillan company, 1910. xvii, 340 p. illus. [Also, 1911.]

FROTHINGHAM, LANGDON.
Laboratory guide for the bacteriologist . . . Philadelphia, W. B. Saunders, 1895. 61 p. 2 pl.

FUERTES, JAMES HILLHOUSE, 1863–
Water and public health. The relative purity of waters from different sources. . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1897. x, 75 p. illus. (incl. plan) 6 fold. diagr.

FULLER, GEORGE WARREN. See Biological studies by the pupils of William Thompson Sedgwick.

GAGE, STEPHEN DE MERITTE. See Biological studies by the pupils of William Thompson Sedgwick.

GALLUP, JOSEPH A.
Sketches of epidemic diseases in the State of Vermont; from its first settlement to the year 1815. With a consideration of their causes, phenomena, and treatment. To which is added remarks on pulmonary consumption . . . Boston, T. B. Wait and sons 1815. 419 p.

GAMALEÏA, N[IKOLAÏ FYODOROVICH], 1859–
The bacterial poisons . . . tr. E. P. Hurd. Detroit, Geo. S. Davis, 1893. 136 p. [The physicians' leisure library.]

GARBAT, ABRAM LEON. See Citron, Julius Bernhard.

GAY, FREDERICK P. See Bordet, Jules.

GEHRMANN, ADOLPH. See . . . Anatomy, physiology, pathology, bacteriology, dictionary, etc., etc.

GIRARD, ALFRED CONRAD, (ed.) See Peyer, Alexander.

GORHAM, FREDERICK POOLE, 1871–1933.
A laboratory course in bacteriology, for the use of medical, agricultural, and industrial students. . . . With 97 illustrations. Philadelphia and London, W. B. Saunders and company, 1901. 1 p. l., 5–192 p. incl. illus., double tab.

—————
A laboratory course in bacteriology, prepared for the use of students in Brown university . . . Providence, R. I., Independent citizen press, 1897. 55 p.

GRADLE, HENRY, 1855–
Bacteria and the germ theory of disease. Eight lectures delivered at the Chicago medical college . . . Chicago, W. T. Keener, 1883. 2 p. l., 219 p.

GRIFFITH, FREDERIC RICHARDSON. See Stoney, Emily Marjory Armstrong.

GROTENFELT, GÖSTA, 1855–1922. [Full name: Karl Gustaf Johannes Grotenfelt]
The principles of modern dairy practice from a bacteriological point of view. . . . Authorized American ed. by F. W. Woll . . . 1st ed. 1st thousand. New York, J. Wiley and sons; 1894. 285 p. illus. [Also, 1895]

—————
————— 3d ed., rev., 1908. vi, 286 p. [Also, 1913]

GRUNDE, MARIE. See Bolduan, Charles Frederick.

GÜELL, EUSEBIO GÜELL Y BACIGALUPI, conde de, 1846?–1918.
Immunity through leucomaines. Translated from the 2d French ed. by R. F. Rafael, M.D. New York, J. H. Vail and co., 1889. x, 170 p.

GUERARD, ARTHUR ROSE. See Park, William Hallock.

HARDOWAY, W. A.

Essentials of vaccination; a compilation of facts relating to vaccine inoculation and its influence in the prevention of small-pox. Chicago, Jansen, McClurg and company, 1882. 146 p.

HARDY, Mrs. E. D. See Belcher, Sarah Drowne.

HARE, HOBART AMORY, 1862–1931, (ed.)

A system of practical therapeutics. Ed. by Hobart Amory Hare ... Assisted by Walter Chrystie ... Philadelphia, Lea brothers and co., 1891–97. 4 v. illus., pl.

———

A text-book of practical therapeutics, with especial reference to the application of remedial measures to disease and their employment upon a rational basis.... Philadelphia, Lea brothers and co., 1890. vi p., 1 l., [17]–632 p.

———

————— 2d ed., enl. and thoroughly rev. 1891. ix p., 1 l., [17]–658 p.

———

————— ...3d ed., enl. and thoroughly rev. 1892. 4 p. l., [xi]–xiii p., 1 l., [17]–696 p.

———

————— ...4th ed., enl. and thoroughly rev. 1894. 11, [17]–740 p.

———

————— ...5th ed., enl. and thoroughly rev. 1895. 11, [17]–740 p.

———

————— ...6th ed., enl., thoroughly rev. and largely re-written. Philadelphia and New York, Lea brothers and co., 1897. 758 p.

———

————— ...7th ed., enl., thoroughly rev. and largely re-written. 1898. 776 p. illus., chart.

———

————— ...8th ed., enl., thoroughly rev. and largely re-written. Illustrated with 37 engravings and 3 colored plates. 1900. 798 p. illus. III col. pl. (incl. diagr.)

———

————— ...9th ed., enl., thoroughly rev. and largely re-written. Illustrated with 105 engravings and 4 colored plates. 1902. 857 p. illus., col. pl., chart.

———

————— ...10th ed., enl., thoroughly rev. and largely re-written. Illustrated with 113 engravings and 4 colored plates. 1904. xi, [17]–908 p. illus., 4 col. pl. (incl. 1 chart).

———

————— ...11th ed., enl., thoroughly rev. and largely re-written. Illustrated with 113 engravings and 4 colored plates. 1905. ix, [17]–910 p. illus., 3 col. pl., col. chart.

———

————— ...12th ed., enl., thoroughly rev. and largely re-written. Illustrated with 114 engravings and 4 colored plates. 1907. ix, [17]–939 p. illus., IV col. pl.

———

————— ...13th ed., enl., thoroughly rev., and largely re-written. Illustrated with 122 engravings and 4 colored plates. Philadelphia and New York, Lea and Febiger, 1909. ix, [17]–958 p. illus., IV col. pl.

———

————— ...14th ed., enl., thoroughly rev., and largely re-written. Illustrated with 131 engravings and 8 plates. 1912. x, [9]–984 p. illus. VIII pl. (partly col.)

———

————— ...15th ed., enl., thoroughly rev., and largely re-written. Illustrated with 144 engravings and 7 plates. 1914. 1 p. l., V–X, [9]–998 p. illus., VII pl. (3 col.) diagrs.

HARE, HOBART AMORY. See Coplin, William Michael Late.

HARRIS, ELISHA, 1824–1884.

Pestilential diseases, and the laws which govern their propagation. A letter from Elisha Harris . . . in reply to inquiries addressed by the quarantine commissioners Transmitted to the Legislature by the governor, March 10, 1858. Albany, C. Van Benthuysen, printer, 1858. 23 p.

HARRIS, NORMAN MACLEOD, (ed.) See Muir, Robert.

Harvard University. *Medical School.*

Directions for laboratory work. Course in bacteriology. Boston, 1902. 18 p.

——— Laboratory directions, Department of bacteriology, Harvard medical school. 4th ed. Boston [⁰1911] 68 p. fold. tab.

——— 5th ed. 1913.

——— 6th ed. 1915.

Harvard University. *Medical school,* Boston, Mass., Department of Bacteriology.

Bacteriology questions for Harvard medical students. n.p., 1902. 9 p.

HASTINGS, EDWIN GEORGE.

A laboratory manual of general agricultural bacteriology, by E. G. Hastings, Conrad Hoffmann, and W. H. Wright. Madison, College of agriculture, University of Wisconsin, 1911. 47 p.

HASTINGS, EDWIN GEORGE. See Russell, Harry Luman.

HATFIELD, MARCUS PATTEN, 1849–

The acute contagious diseases of childhood . . . Chicago, G. P. Engelhard and company, 1901. 3 p. l., [9]–135, vi p. illus. [Standard monograph series]

HAYES, MATTHEW HORACE, 1842–

Notes and appendix to the fourth edition of Friedberger and Fröhner's Lehrbuch der speciellen pathologie und therapie der hausthiere. By M. H. Hayes . . . with a chapter by Dr. G. Newman, D. P. H. London, W. Thacker and co., 1898. 68 p. ["Chaper IV. By George Newman . . . Notes on bacteriology."]

HEALY, WILLIAM. See . . . Anatomy, physiology, pathology, bacteriology, dictionary, etc., etc.

HEINEMANN, PAUL GUSTAV.

A laboratory guide in bacteriology, for the use of students, teachers, and practitioners . . . Chicago, The University of Chicago press, 1905. xiv, 143 p. illus., diagrs. [Also, 1911, 210 p.]

——— 2d ed. Chicago, Ill., The University of Chicago press [⁰1913] xv, 210 p. illus., diagrs. (1 fold.)

HEKTOEN, LUDVIG, 1863– (ed.)

An American text-book of pathology, for the use of students and practitioners of medicine and surgery, ed. by Ludvig Hektoen . . . and David Riesman, 1867, . . . with 443 illustrations, 66 of them in colors. Philadelphia and London, W. B. Saunders and company, 1901. 1 p. l., [9]–1245 p. illus.

HERRICK, JAMES B.

A handbook of medical diagnosis for students . . . 80 illustrations and 2 colored plates. Philadelphia, Lea brothers and co., 1895. 432 p.

HERSEY, HARVEY.

Our friends and our foes of the invisible world; how to woo the friends; how to conquer the foes . . . with one hundred and sixteen illustrations. New York, The Neale publishing company, 1913. 6 p. l., [3]–349 p. illus , plates (part col.) diagrs.

HERTER, CHRISTIAN ARCHIBALD, 1865–1910.

The common bacterial infections of the digestive tract and the intoxications arising from them . . . New York, the Macmillan company; London, Macmillan and co., ltd.,

1907. x p., 1 1., 360 p. ["This little volume embodies views recently presented at the New York Academy of medicine, in a lecture before the Harvard society for the diffusion of medical knowledge."—Pref.]

HERZOG, MAXIMILIAN JOSEPH, 1858–

A text-book on disease-producing microörganisms, especially intended for the use of veterinary students and practitioners ... with 214 illustrations in black and 14 colored plates. Philadelphia and New York, Lea and Febiger, 1910. xi, [17]–644 p. illus., XIV col. pl. (incl. front.)

HISS, PHILIP HANSON, 1868?–1913.

A text-book of bacteriology, a practical treatise for students and practitioners of medicine, by Philip Hanson Hiss, Jr. ... and Hans Zinsser, 1898–1940 ... with one hundred and fifty-six illustrations in the text, some of which are colored. New York and London, D. Appleton and company, 1910. xiv, 745 p. illus. [Also, 1911]

———— ... with one hundred and fifty-six illustrations in the text, some of which are colored. 2d ed. 1914. xvi, 766 p. illus. (part col., incl. chart) [Also, 1915]

HOBBS, GLENN MOODY. See American school of correspondence, Chicago, 1909.

HOFFMAN, CONRAD. See Hastings, Edwin George.

HOLMES, HORACE PERRY. See Jousset, Pierre.

HOLT, L. EMMET. See Flexner, Simon.

HOSACK, DAVID, 1769–1835.

Observations on febrile contagion, and on the means of improving the medical police of the city of New York. Delivered as an introductory discourse, in the hall of the College of physicians and surgeons, on the sixth of November, 1820 ... New York: Published by Elam Bliss, no. 208 Broadway. J. Seymour, printer, 1820. 3 p. l., [3]–79 p.

HOWELL, STEPHEN YATES, (tr.) See Friedlaender, Carl.

HUEPPE, FERDINAND ADOLPH THEOPHIL, 1852–

The methods of bacteriological investigation.... Tr. by Hermann M. Biggs ... Illustrated by thirty-one wood-cuts. New York, D. Appleton and company, 1886. 3 p. l., [5]–218 p. illus.

———— The principles of bacteriology, by Dr. Ferdinand Hueppe ... authorized translation from the German by Dr. E. O. Jordan ... Chicago, The Open court publishing company; London, K. Paul, Trench, Trübner and co., 1899. 3 p. l., V–X, 467 p. col. front., illus., plates (part col.)

HURD, EDWARD PAYSON, (tr.) See Liebermeister, Karl von.

———— See Trouessart, Édouard Louis.

IRELAND, G. H. See American public health association, 1886.

IVY, ROBERT HENRY. See Thomas, Benjamin Abraham.

JACKSON, DANIEL DANE. See Biological studies by the pupils of William Thompson Sedgwick.

JACOBI, ABRAHAM, 1830–

A treatise on diphtheria. By A. Jacobi ... New York, W. Wood and co., 1880. x p., 1 1., 252 p.

JAMESON, HORATIO GATES, 1778–1855.

Observations intended to prove the non-contagious nature of yellow fever, and that the different forms of bilious, and yellow or malignant fever are produced by morbific exhalations, arising from marshes, or vegetables in other situations, undergoing the putrefactive fermentation ... Baltimore, Printed at the Morning chronicle office, 1823. 46 p.

JANEWAY, HENRY HARRINGTON, 1873–

Lecture notes on bacteriology, for dental students. New York, P. B. Hoeber [c1915] 216 p. illus.

JENNER, ANDREW J. B.

Disinfection, including personal prophylaxis against infectious and contagious diseases ... Detroit, J. F. Eby and company, printers, 1886. vii, [9]-128 p.

JORDAN, EDWIN OAKES, 1866-1936.

A text-book of general bacteriology ... Philadelphia and London, W. B. Saunders company, 1908. 2 p. l., [11]-557 p. illus., 2 fold. charts. [Also, 1909]

———— ———— 2d ed., thoroughly rev. 1910. 2 p. l., [9]-594 p. illus., fold. map, fold. tab.

———— ———— 3d ed., thoroughly rev. 1912. 2 p. l., 9-623 p. illus., fold. chart, fold. tab. [Also, 1913]

———— ———— 4th ed., thoroughly rev. 1914. 2 p. l., [9]-647 p. illus. (part col.) 2 fold. charts. [Also, 1915]

JORDAN, EDWIN OAKES. See Biological studies by the pupils of William Thompson Sedgwick.

———— See Chicago University.

———— See Hueppe, Ferdinand Adolph Theophil.

JOUSSET, PIERRE, 1818-

The pathogenic microbes by M. le Dr. P. Jousset ... Authorized translation of Horace P. Holmes, M.D. Philadelphia, Boericke and Tafel, 1903. 192 p.

JOWETT, WALTER

Notes on blood-serum therapy, preventive inoculation and toxin and serum diagnosis for veterinary practitioners and students ... Chicago, W. T. Keener and co., 1907. viii, 204 p. incl. illus., plates.

KENDALL, ARTHUR ISAAC. See Biological studies by the pupils of William Thompson Sedgwick.

KLEMPERER, FELIX. See Levy, Ernst.

KLOPSTOCK, MARTIN.

A manual of clinical chemistry, microscopy, and bacteriology, by Dr. M. Klopstock and Dr. A. Kowarsky ... Only authorized translation from the last German ed. thoroughly rev. and enl.; illustrated with forty-three textual figures and sixteen colored plates. New York, Rebman company [°1912] 10 p. l., 371 p. illus., XVI col. pl.

KOLMER, JOHN ALBERT, 1886-

A practical text-book of infection, immunity and specific therapy, with special reference to immunologic technic ... with an introduction by Allen J. Smith ... With 143 original illustrations, 43 in colors, by Erwin F. Faber ... Philadelphia and London, W. B. Saunders company, 1915. xi, [3]-899 p. illus., col. plates.

KORNITZER, JOSEPH.

The pathology and abortive treatment of all zymotic and inflammatory diseases. An essay by Dr. J. Kornitzer ... [Cincinnati, O., °1880] 27, [1] p.

KOWARSKY, ALBERT. See KLOPSTOCK, MARTIN.

LANGFELD, MILLARD, 1872-

Introduction to infectious and parasitic diseases, including their cause and manner of transmission ... With an introduction by Lewellys F. Barker ... with thirty-three illustrations. Philadelphia, P. Blakiston's son and co., 1907. xvi, 260 p. illus., pl.

Lederle antitoxin laboratories. *Medical department.*

Modern biologic therapeusis; a concise and practical treatise on biologic products for the use of practitioners in the modern application of immunology to therapeutics ... New York, Medical department of the Lederle antitoxin laboratories, 1915. 3 p. l., ix-xx, 322 p. illus. (incl. ports.)

LEFFMANN, HENRY, 1847-

Examination of water for sanitary and technical purposes. *By Henry Leffmann* ... and William Beam ... 2d ed., rev. and enl., with illustrations. Philadelphia, P. Blakiston, son and company, 1891. vii, [9]-130 p. incl. illus., tables.

Examination of water for sanitary and technic purposes. By Henry Leffmann ... 3d ed., rev. and enl., with illustrations. 1895. xi, [1], 13–154 p. illus., diagrs.

————4th ed., rev. and enl. ... 1899. 2 p. l., vii–xi, 13–145 p. illus.

————5th ed., rev. and enl. ... 1903. 2 p. l., vii–xi, 13–140 p. illus., diagrs.

Examination of water for sanitary and technic purposes, by chemical and bacteriologic methods. 6th ed., rev. and enl., with instructions. 1909. 4 p. l., 144 p. illus., diagrs.

Examination of water for sanitary and technic purposes. 7th ed., rev. and enl., with illustrations ... [*1915] xvi, 140 p. illus.

Select methods in food analysis, by Henry Leffmann ... and William Beam ... with fifty-three illustrations in the text, four full-page plates and many tables. Philadelphia, P. Blakiston's son and co., 1901. 1 p. l., v–viii, 9–383 p. illus., plates.

LEHMANN, KARL BERNHARD, 1858–
    Atlas and principles of bacteriology and textbook of special bacteriologic diagnosis, by Prof. Dr. K. B. Lehmann ... and R. O. Neumann ... Authorized translation from the 2d enl. and rev. German ed. Edited by George H. Weaver ... Philadelphia and London, W. B. Saunders and company, 1901. 2 v. illus., 69 col. pl., fold. tab. (*On cover:* Saunders' medical hand atlases).

LEIGHTON, MARSHALL ORA. See Biological studies by the pupils of William Thompson Sedgwick.

LENHARTZ, HERMANN, 1854–
    Manual of clinical microscopy and chemistry, prepared for the use of students and practitioners of medicine, by Dr. Hermann Lenhartz ... authorized translation from the 4th and last German ed., with notes and additions by Henry T. Brooks ... with 148 illustrations in the text and 9 colored plates ... Philadelphia, F. A. Davis company, 1904. 2 p. l., ix–xxix, 412 p. front., illus., 9 col. pl.

LENTZ, CHAS. AND SONS.
    Catalogue of microscopes and accessories, microtomes, bacteriological apparatus, laboratory supplies and instruments for clinical diagnosis. 5th ed. Chas. Lentz and sons, 18 and 20 North Eleventh St., Philadelphia, 1899. 136 p. illus. [The first complete catalog of bacteriological apparatus published in Philadelphia. Compiled, written and edited by Simeon Trenner.]

LEVY, ERNST, 1864–
    Elements of clinical bacteriology for physicians and students, by Dr. Ernst Levy ... and Dr. Felix Klemperer ... 2d enl. and rev. ed. Authorized translation by Augustus A. Eshner ... Philadelphia, W. B. Saunders, 1900. 1 p. l., [7]–441 p. illus., col. pl.

LEVY, ERNEST COLEMAN, 1868–
    Bacteriological report of mechanical filtration plant at Danville, Virginia. Pittsburgh, Pittsburgh filter manufacturing company, 1905. 6 p., 1 l.

    Bacteriological report of mechanical filtration plants at Danville, Virginia, and Louisberg, N. C. Pittsburgh, Pa., 1906.

LIEBERMEISTER, KARL VON, 1833–1901.
    Pathology and treatment of the infectious diseases ... Tr. by E. P. Hurd ... with notes and appendices. Detroit, Mich., G. S. Davis. 1888. 2 v. diagrs. [Physicians' leisure library, no. 8] [Part 2 has title: Iinfectious diseases. Paged continuously. Original German ed. published 1885 under title: Infectionskrankheiten, being v. 1 of the author's Vorlesungen über die specielle Pathologie und Therapie.]

LINCOLN. D. F. See American public health association, 1886.

LINSLEY, JOSEPH HATCH, (*ed.* and *tr.*). See Fraenkel, Carl.

LIPMAN, JACOB GOODALE, 1874–

Bacteria in relation to country life ... New York, the Macmillan company, 1908. xx, 486 p. incl. front. (port.) illus. (*On cover:* The Rural science series, by L. H. Bailey)

LOCHRIDGE, E. E. See Biological studies by the pupils of William Thompson Sedgwick.

LOGAN, CORNELIUS AMBROSE, 1836–1899.

Physics of the infectious diseases. Comprehending a discussion of certain physical phenomena in connection with the acute infectious diseases ... Chicago, Jansen, McClung and company, 1878. 2 p. l., [3]–212 p. front. (map.)

McCAMPBELL, EUGENE FRANKLIN.

Laboratory methods for the experimental study of immunity ... Columbus, O., The F. J. Heer printing co., 1909. 197 p. [Pages 13–197 on verso of leaf only].

McCAMPBELL, EUGENE FRANKLIN. See Frost, William Dodge.

MacCAUGHEY, VAUGHAN, 1887–

Identification tables for certain important bacteria. List of bacteria producing important plant diseases, prepared by Vaughan MacCaughey ... for use in the courses in bacteriology. [Honolulu, The College, 1910] 16 p.

McCONNELL, GUTHRIE, 1875–

Pathology and bacteriology for dental students ... Philadelphia and London, W. B. Saunders company, 1915. 2 p. l., [13]–309 p. illus., col. pl.

McFARLAND, JOSEPH, 1868–

A text-book upon the pathogenic *Bacteria* ... With 113 illustrations. Philadelphia, W. B. Saunders, 1896. 2 p. l., [11]–359 p. col. front., illus.

—————

A text-book upon the pathogenic *Bacteria*; for students of medicine and physicians, by Joseph McFarland ... With 142 illustrations. 3d ed., rev. and enl. Philadelphia, W. B. Saunders and company, 1900. 621 p. incl. col. front., illus. plates.

—————

A text-book upon the pathogenic bacteria, for students of medicine and physicians ... with 153 illustrations, a number of them in colors. 4th ed., rewritten and enl. Philadelphia, London etc. W. B. Saunders and company, 1903. 2 p. l., 7–629 p. illus. (partly col.) 2 pl., tab.

—————

————— ... With 190 illustrations, a number of them in colors. 5th ed., thoroughly rev. Philadelphia and London, W. B. Saunders company, 1906. 2 p. l., 7–647 p. illus. (part col.) II pl., chart. [Also, 1907]

—————

————— ... with 211 illustrations, a number of them in colors. 6th ed., thoroughly rev. Philadelphia and London, W. B. Saunders company, 1909. 709 p. illus. (part col.) III pl., fold. tab.

—————

————— A text-book upon the pathogenic bacteria and protozoa, for students of medicine and physicians ... with 293 illustrations, a number of them in colors. 7th ed., thoroughly rev. Philadelphia and London, W. B. Saunders company, 1912. 878 p. illus. (partly col.) II col. pl., chart.

—————

A text-book upon the pathogenic *Bacteria* and *Protozoa* for students of medicine and physicians. 8th ed., rev., with 323 illustrations, a number in colors. 1915. 3 p. l., 13–807 p. illus. (part col.) III col. pl., fold. chart., *diagr.*

McFARLAND, JOSEPH. See Carter, William S. (*comp.*)

MacFARLANE, ANDREW.

A clinical manual; a guide to the practical examination of the excretions, secretions, and the blood, for the use of physicians and students ... New York [etc.] G. P. Putnam's sons, 1894. xii, 139 p. illus., col. plates.

McKail, David.

Public health chemistry and bacteriology; a handbook for D. P. H. students. New York, 1912. 409 p.

McIsaac, Isabel.

Bacteriology for nurses . . . New York, The Macmillan company, 1909.   xii, p., 1 l., 179 p. illus.

———— . . . 2d ed. Rev. New York, The Macmillan company, 1914.   xii p., 1 l., 179 p. illus.

McLaughlin, James Wharton, 1840–

Fermentation, infection and immunity. A new theory of these processes, which unifies their primary causation and places the explanation of their phenomena in chemistry, biology, and the dynamics of molecular physics . . . Austin, Tex., E. Von Boeckmann, printer, 1892.   240 p.

MacNeal, Ward J. 1881–

Pathogenic micro-organisms; a text-book of microbiology for physicians and students of medicine . . . (Based upon Williams' Bacteriology) with 213 illustrations.   Philadelphia, P. Blakiston's sons and co., 1914.   xxi, 462 p. illus., fold. chart.

Magnin, Antoine.   [*Full name:* Antoine Marie Magnin]

The *Bacteria*.   By Dr. Antoine Magnin . . . Translated by George M. Sternberg . . . Boston, Little, Brown, and company, 1880.   227 p. x pl.

Bacteria. By Dr. Antoine Magnin . . . and George M. Sternberg . . . New York, W. Wood and company, 1884.   xviii p., 1 l., [11]–494 p. illus., xii pl. [The original work, Les bactéries, was first published in Paris, 1878, and translated by Dr. Sternberg in 1880.   To the present edition the translator has added: pt. III. Technology, pt. IV. Germicides and antiseptics, pt. V. *Bacteria* in infectious diseases and pt. VI. *Bacteria* in surgical lesions.   Pt. I-II on the morphology and physiology of the *Bacteria* have not been rewritten.   *cf.* Pref.]   [Also, 1885, 488 p.]

Bacteria. By Magnin, Antoine [Marie] and Sternberg, George M. 2d ed.   New York, W. Wood and company, 1885.   488 p.

Mallory, Frank Burr, 1862–

The principles of pathologic histology, by Frank B. Mallory . . . with 497 figures containing 683 illustrations, 124 in colors, and all but two original printed directly in the text.   Philadelphia and London, W. B. Saunders company, 1914.   2 p. l., 11–677 p. illus. (part col.)

Pathological technique; a practical manual for the pathological laboratory, by Frank Burr Mallory . . . and James Homer Wright . . . Philadelphia, W. B. Saunders, 1897. 2 p. l., 11–397 p. illus.

Pathological technique; a practical manual for workers in pathological histology and bacteriology . . . By Frank Burr Mallory . . . and James Homer Wright . . . 2d ed. rev. and enl. . . . Philadelphia, London, W. B. Saunders and company, 1901. 2 p. l., 9–432 p. illus. (part col.) pl.

Pathological technique; a practical manual for workers in pathological histology and bacteriology including directions for the performance of autopsies and for clinical diagnosis by laboratory methods, by Frank Burr Mallory and James Homer Wright . . . 3d ed., rev. and enl., with 156 illustrations.   Philadelphia [etc.] W. B. Saunders and company, 1904.   2 p. l., 9–469 p. illus., plates.

———— 4th ed., rev. and enl., with 152 illustrations.   Philadelphia and London, W. B. Saunders company, 1908.   2 p. l., 9–480 p. illus. (part col.) 2 pl.

————5th ed., rev. and enl., with 162 illustrations. 1911. 2 p. l., 9–507 p. illus. (part col.) 2 pl.

———— 6th ed., rev. and enl., with 174 illustrations. 1915. 2 p. l., 9–536 p. illus. (part col.) plates.

MALLORY, FRANK BURR. See Councilman, William Thomas.
———— See Nuttall, G. H. F.

MARCHIAFAVA, ETTORE, 1847–
Malaria and micro-organisms. By Ettore Marchiafava . . . Amico Bignami . . . Simon Flexner . . . Eugene L. Opie . . . Specially reprinted from "Twentieth century practice," for the use of the United States government. New York, W. Wood and company, 1900. 3 p. l., 828 p. illus., xi pl.

MARSHALL, CHARLES EDWARD, 1866–1927, (ed.)
Microbiology for agricultural and domestic science students. Contributors: F. T. Bioletti . . . R. E. Buchanan . . . [etc.] ed. by Charles E. Marshall . . . with 128 illustrations. Philadelphia, P. Blakiston's son and co., 1911. xxi, 724 p. illus., col. pl. [Also, 1912]

Bacteriology and the bacteriological laboratory. n.p., 1904. 27 p.

MASON, WILLIAM PITT, 1853–
Examination of water. (Chemical and bacteriological.) 1st ed. 1st thousand. New York. J. Wiley and sons; [etc., etc.] 1899. 3 p. l., 135 p. incl. illus., 2 pl., diagrs. 2 fold. maps.

———— 2d ed., rev. 1st thousand. New York, J. Wiley and sons; London, Chapman and Hall, limited, 1901. 3 p. l., 131 p. incl. illus., 2 pl., 2 fold. maps.

————3d ed., rev. New York. J. Wiley and sons; etc., etc. 1906. v. 155 p. incl. illus., plates, diagrs.

———— 4th ed., rev. 1st thousand. 1910. iii p., 1 l., 167 p. incl. illus., plates. fold. maps.

Water-supply. (Considered principally from a sanitary standpoint.) 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1896. vii, 504 p. front., illus., 2 fold. maps, diagrs. [Also, 1899]

———— 3d ed., rewritten. 1st thousand. 1902. vii, 448 p. incl. illus., plates, diagrs. front., plates, fold. map. [Also, 1906]

MEAD, D. W. See Turneaure, F. E.

MEARS, JAMES EWING, 1838–
Is there danger of the conveyance of disease through the use of the cup in the communion service? . . . An address delivered before the Woman's guild of Trinity church parish, St. Augustine, Florida, January 31, 1910. New York, T. Whitaker, inc. [1910] 16 p.

Mexico. *Ministerio de instrucción pública y bellas artes.*
Howard Taylor Ricketts y sus trabajos sobre el tabardillo (tifo de México) Publicado por la Secretaría de instrucción pública y bellas artes en cumplimiento del acuerdo relativo del presidente de la república. Mexico, Tip. de la vda. de F. Diaz de Leon, sucs., 1910. 2 p. l., 137 p. front. (port.) 17 charts, fold tab.

MILLER, FRANK WILLIAM, 1866–
Fundamentals of physics, chemistry and bacteriology in agriculture. Columbus, O., The F. J. Heer printing co., 1913. 48 p. illus.
["Issued by the Department of public instruction of the state of Ohio."]

MILLER, WILLOUGHBY DAYTON, 1853–1907.

Die mikroorganismen der mundhöhle. Die örtlichen und allgemeinen erkrankungen, welche durch dieselben hervorgerufen werden . . . Mit 112 abbildungen im texte und einer chromolithographischen tafel. Leipzig, G. Thieme, 1889. xx, 305, [1] p. illus., col. pl.

————— 2ᵉ umgearb. und erweiterte aufl. 1892. 448 p.

The micro-organisms of the human mouth. The local and general diseases which are caused by them . . . With one hundred and twenty-eight illustrations, one chromo-lithographic and two photo-micrographic plates. Philadelphia, The S. S. White dental mfg. co., 1890. 1 p. l., [v]-xx p., 1 l., 364 p. illus., III pl. (1 col.)

MILLIKEN, CARL SPENCER. See Biological studies by the pupils of William Thompson Sedgwick.

MITCHELL, JOHN KEARSLEY, 1798–1858.

Five essays. By John Kearsley Mitchell . . . Ed. by S. Weir Mitchell . . . Philadelphia, J. B. Lippincott and co., 1859. xiv, [13]-371 p. illus. [*Contents:* Essay upon the cryptogamous origin of malarious and epidemic fevers.—An essay upon animal magnetism, or vital induction.—On the penetrativeness of fluids.—On the penetrativeness of gases.—On a new practice in acute and chronic rheumatism.]

On the cryptogamous origin of malarious and epidemic fevers . . . Philadelphia, Lea and Blanchard, 1849. viii, [13]-137 p.

MITCHELL, S. WEIR. See Mitchell, John Kearsley.

MOORE, VERANUS ALVA, 1859–1931.

. . . Bacteria in milk. A summary of the present knowledge concerning their source and significance. Albany, J. B. Lyon company, state printers, 1902. 28 p. III pl., v. diagr. (1 fold.) [At head of title: State of New York—Department of agriculture. "References": p. [25]-26.]

Bovine tuberculosis and its control . . . thirty full page illustrations. Ithaca, N. Y., Carpenter and company, 1913. x, 134 p., 1 l. front. (port.) XXX pl.

Laboratory directions for beginners in bacteriology . . . Ithaca, N. Y., Press of Andrus and Church, 1898. vi, [2] p., 1 l., [11]- 89 p.

Laboratory directions for beginners in bacteriology; an introduction to practical bacteriology for students and practitioners of comperative and of human medicine. 2d ed., enl. and rev. Boston, U. S. A., Ginn and company, 1900. xvi, 143 p. front., illus.

————— 3d ed., enl. and rev. Boston, London [etc.] Ginn and company [1905] xxiii, 151 p. front., illus., diagr.

The pathology and differential diagnosis of infectious diseases of animals, by Veranus Alva Moore . . . with an introduction by Daniel Elmer Salmon . . . Ithaca, N. Y., Taylor and Carpenter, 1902. xiv, 380 p. front., illus., pl. (part col.) map, diagrs.

————— 2d ed. rev. and enl. 1906. xvi, 506 p. illus., IX pl. (incl. map, 2 col.)

————— 3d ed., rev. and enl. with 127 illustrations, 1908. xvi, 578 p. illus. II pl. (1 col.)

The presence of septic bacteria, probably identical with those of swine plague, in the upper passages of domesticated animals other than swine. (*In* Smith, Theobald.

Special report on the cause and prevention of swine plague. Appendix. p. 151–159. Washington, 1891).

———

Principles of microbiology; a treatise on *Bacteria, Fungi* and *Protozoa* pathogenic for domesticated animals ... one hundred and one illustrations. Ithaca, N. Y., Carpenter and company, 1912. xi, 506 p. illus.

———

Exercises in bacteriology and diagnosis for veterinary students and practitioners, by Veranus A. Moore ... and Clifford P. Fitch ... Boston, New York etc. Ginn and company [c1914] xix p., 1 l., 154 p. illus., diagr.
[On cover: Bacteriology and diagnosis. 1898, 1900 and 1905 editions published under title: Laboratory directions for beginners in bacteriology, by Veranus A. Moore.]

MUIR, ROBERT, 1864–

Manual of bacteriology, by Robert Muir ... and James Ritchie ... American edition (with additions) rev. and ed. from the 3d English edition, by Norman MacLeod Harris ... with one hundred and seventy illustrations. New York. The Macmillan company; London, Macmillan and co., ltd., 1903. xx p., 1 l., 565 p. illus., diagr.

MULFORD, H. K. COMPANY.

The present status of diphtheria antitoxic serum. Philadelphia and Chicago, The Company, 1897. 40 p.

———

Rabies vaccine prepared after the method of Pasteur for the preventive treatment of rabies. Philadelphia, New York [etc.] H. K. Mulford company [1911?] 23 p. illus.

———

A treatise on bacterins (bacterial vaccines). Theories of immunity and of bacterial therapy. From a general standpoint and intended for the general practitioner Philadelphia. The Company, 1911. 81 p. [Mulford Working Bulletin, no. 1.]

NEUMANN, R. O. See Lehmann, Karl Bernhard.

NEWMAN, GEORGE. See Hayes, Matthew Horace.

NORTON, JOHN F. See Wodman, Alpheus Grant.

NOVY, FREDERICK GEORGE, 1864–

Directions for laboratory work in bacteriology ... Ann Arbor, Mich., G. Wahr [1894] 209 p. front.

———

——— 2d ed., rev. and enl., with frontispiece and seventy-six illustrations. Ann Arbor, [Mich.], G. Wahr, 1899. 563 p. front., illus.

NOVY, FREDERICK GEORGE. See Vaughan, Victor Clarence.

NUTTALL, GEORGE HENRY FALKINER, 1862–   (ed.)

The bacteriology of diphtheria including sections on the history, epidemiology and pathology of the disease, the mortality caused by it, the toxins and antitoxins and the serum disease, by F. Loeffler ... Arthur Newsholme ... F. B. Mallory ... G. S. Graham-Smith ... George Dean ... William H. Park ... Charles F. Golduan [Bolduan] ... Ed. by G. H. F. Nuttall ... and G. S. Graham-Smith ... Cambridge, University press, 1908. xx, 718 p. illus., XVI pl., 4 port., diagrs. (1 fold)

———

Hygienic measures in relation to infectious diseases ... New York [etc.] G. P. Putnam's sons, 1893. xi, 112 p.

OPIE, EUGENE L. See Marchiafava, Ettore.

OSBORNE, OLIVER THOMAS, 1862–

The prevention and treatment of infections, by Oliver T. Osborne ... Chicago, The Journal of the American medical association [c1915] 3 p. l., 239 p.

OSBURN, WILLIAM.

Manual of histology and bacteriology; including a concise statement of the important facts of microscopic technique and urinalysis, and a laboratory course of seventy practical exercises, with provision for notes and drawings ... Nashville, Tenn., Marshall and Bruce company, 1899. 188, [4] p. illus.

O'SHEA, M. V. See Frost, William Dodge.

OSLER, *Sir* WILLIAM, *bart.*, 1849–1919.

    ... Bacilli and bullets, by Sir William Osler ... 4th impression ... London, New York [etc.] Oxford University press, H. Milford [1914] 8 p. (Oxford Pamphlets, 1914 [no. 30]).

ORCUTT, I. H.

    Microbes and men ... Owatonna, Minn., For sale by the author, 1894. 136 p. incl. front. (port.) illus. diagrs.

Palisade Manufacturing Company, Yonkers, N. Y.

    Syllabus of bacteriology. A compact treatise designed to aid the physician in the microscopic diagnosis of disease. Yonkers, The Company, 1901. 45 p. illus.

PALMER, W. C.

    Farm bacteriology ... Winona Lake, Indiana, 18 p., 1908.

PARK, WILLIAM HALLOCK, 1863–1939.

    Bacteriology in medicine and surgery. A practical manual for physicians, health officers, and students. By Wm. Hallock Park ... assisted by A. R. Guerard ... New York and Philadelphia, Lea brothers and co. [1899] xi, [17]–693 p. illus., II col. pl. [Subsequently published under title: Pathogenic microorganisms.]

    Pathogenic micro-organisms, including bacteria and *Protozoa;* a practical manual for students, physicians and health officers, by William Hallock Park ... assisted by Anna W. Williams ... 2d ed., enl. and thoroughly rev. With 165 engravings and 4 full-page plates. New York and Philadelphia, Lea brothers and co., 1905. vii, [17]–556 p. illus., IV pl. (part col.)

    ... 3d ed., enl. and thoroughly rev., with 176 engravings and 5 full-page plates. New York and Philadelphia, Lea and Febiger, 1908. viii, 642 p. illus., V pl. (4 col.)

    ———— 4th ed., enl. and thoroughly rev., with 196 engravings and 8 full-page plates. 1910. viii, 670 p. illus., VIII pl. (7 col.) fold. tab.

    ———— 5th ed., enl. and thoroughly rev., with 210 engravings and 9 full-page plates. 1914. viii, [17]–684 p. illus., IX pl. (part col.) tables (1 fold.) diagrs.

PARK, WILLIAM HALLOCK. See Belcher, Sarah Drowne.

———— See Nuttall, G. H. F.

PEEBLES, ISAAC LOCKHART.

    Unanswerable objections to vaccination. Fourth thousand. Nashville, Tennessee, Publishing House. M. E. Church, South. 1914. 83 p.

PEYER, ALEXANDER.

    An atlas of clinical microscopy, by Alexander Peyer, M.D. Tr. and ed. by Alfred C. Girard ... 1st American, from the manuscript of the 2d German ed., with additions. Ninety plates, with one hundred and five illustrations ... New York, D. Appleton and company, 1885. xiv, 194 p. 90 pl. (part col.)

PFINGST, ADOLPH O.

    Manual of elementary bacteriology, by Adolph O. Pfingst ... and John E. Cashin ... Louisville, Ky., J. P. Morton and company, 1898. 2 p. 1., [vii]–xvi, 187 p. illus. (part col.)

PHELPS, EARLE BERNARD. See Biological studies by the pupils of William Thompson Sedgwick.

A physical enquiry into the origin and causes of the pestilential fevers ... New-York: Printed by J. Tiebout, for Thomas B. Jansen, bookseller and stationer, no. 344 Water-street, near New-slip. 1798. 32 p.

PIERCE, NORVAL HARVEY. See Eisenberg, James.

PINA Y CASAS, ENRIQUE.

    Venenos zymóticos ... Puerto-Rico, Impr. del Boletin mercantil, 1884. 29 p.

PITFIELD, ROBERT LUCAS, 1870–

   . . . A compend on bacteriology, including animal parasites . . . with 4 plates and 80 other illustrations. Philadelphia, P. Blakiston's son and co., 1907. vii, 232 p. illus., IV pl. [Blakiston's? quiz-compends?]

—————— 2d ed. with 4 plates and 85 other illustrations. 1913. vii, 280 p. inc. illus. (part col.) IV pl. [Blakiston's? quiz-compends?]

POTTER, THEODORE, 1816–1915.

   Essays on bacteriology and its relation to the progress of medicine. Indianapolis, Indiana, Medical journal publishing co., 1898. 161 p.

POULTON, EDWARD BAGNALL, 1856–

   . . . A remarkable American work upon evolution and the germ theory of disease. The presidential address read before the Linnean society of London at the anniversary meeting, May 24, 1913, by Professor Edward B. Poulton. London, Printed by Taylor and Francis, 1913. [1], 26–45 p. ["Extracted from the Proceedings of the Linnean society of London, session 1912–13." An address on the authenticity of the work "Shall we have common sense. Some lectures written and delivered by Geo. W. Sleeper. Boston, Wm. Bense, printer, 1849," including a reproduction of the title page and quotations from the work.]

—————— . . . A remarkable American work upon evolution and the germ theory of disease. By Professor Edward B. Poulton . . . Addresses delivered at the anniversary meetings of the Linnean society of London on the 24th of May, 1913, and the 25th of May, 1914. London, Printed by Taylor and Francis, 1913–1914. cover-title, [1], 26–45 p., 1 l., 22, 2 p., 2 l., 36 p. facsim. [The addresses have each separate t.-p. and are extracted from the Proceedings of the Linnean society of London, session 1912–13, 1913–14. They are on the authenticity of the work "Shall we have common sense. Some recent lectures written and delivered by Geo. W. Sleeper. Boston, Wm. Bense, printer, 1849."]

PRESCOTT, SAMUEL CATE, 1876–

   . Elements of water bacteriology, with special reference to sanitary water analysis. By Samuel Cate Prescott . . . and Charles-Edward Amory Winslow . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1904. x, 162 p.

—————— . . . 2d ed., rewritten. 1st thousand. 1908. xii, 258 p. incl. illus., tables.

—————— . . . 3d ed., rewritten. 1st thousand. 1913. xiv, 318 p. illus.

PRESCOTT, SAMUEL CATE. See Biological studies by the pupils of William Thompson Sedgwick.

PRICE, GEORGE M.

   Handbook on sanitation. A manual of theoretical and practical sanitation. For students and physicians; for health, sanitary, tenement-house, plumbing, factory, food, and other inspectors; as well as for candidates for all municipal positions. First ed., first thousand. New York, John Wiley and sons, 1901. 11 and 317 p. illus.

—————— 2d ed. rev. and partly rewritten. 1905. 14 and 301 p.

—————— 3d ed. rewritten and reset. 1913. 11 and 353 p.

PRUDDEN, THEOPHIL MITCHELL, 1849–1924.

   Drinking-water and ice supplies and their relations to health and disease . . . New York etc. G. P. Putnam's sons, 1891. v p., 1 l., 148 p. illus., pl.

—————— Dust and its dangers . . . 2d ed., illustrated. New York and London, G. P. Putnam's sons, 1910. 5 p. l., 113 p. illus., plates.

The story of the bacteria and their relations to health and disease. New York and London, G. P. Putnam's sons, 1889. 3 p. l., 143 p. [Also, 1890, 1891.]

———— 2d ed., rev. and enl. 1910. x p., 1 l., 232 p. illus., plates.
PRUDDEN, THEOPHIL MITCHELL. See Delafield, Francis.
PUTNAM, JOHN J.
The bacteria of Nebraska soil; with special reference to the fixation of nitrogen, ammonification, denitrification in non-protein media, including observations on the reduction of nitrates by soil bacteria in general . . . Lincoln, Neb., Woodruff press, 1913. 54 p. plates. [Also, Thesis—University of Nebraska, 1914]
RADAM, WILLIAM.
Microbes and the microbe killer, by William Radam . . . New York, The author, 1890. xiii, 369 p. front. (port.) plates.

———— Rev. ed. New York, The author, 1895. xviii, 192 p. front. (port.) illus., XXV pl.
RAFAEL, R. F. (tr.) See Güell, Eusebio Guell y Bacigalupi, *conde* de.
REED, HOWARD SPRAGUE, 1876–
A manual of bacteriology for agricultural and general science students . . . Boston, New York etc. Ginn and company [ᶜ1914] xii, 179 p. illus., tables, diagrs.
REEVES, JAMES EDMUND, 1829–
A hand-book of medical microscopy . . . Philadelphia, P. Blakiston, son and co., 1894. xv, 17–237 p. illus. (part col.)

How to work with the Bausch and Lomb optical Co.'s microtome, and a method of demonstrating the tubercle-bacillus . . . Rochester, N. Y., Bausch and Lomb optical company, 1886. 27 p.
REID, MARY ELIZA.
Bacteriology in a nutshell; a primer for junior nurses; comp. and arranged by Mary E. Reid . . . Cincinnati, O., 1904.
RICHARDS, ELLEN HENRIETTA (Swallow) "*Mrs*. R. H. Richards," 1842–1911.
Air, water, and food from a sanitary standpoint. By Ellen H. Richards and Alpheus G. Woodman . . . 1st ed., 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1900. 2 p. l., 226 p. incl. illus., pl., tables. fold. map.

———— . . . 2d ed., rev. and enl. 1st thousand. 1904. 3 p. l., 262 p. illus., pl., fold. map, diagrs.

———— . . . 3d ed., rev. and enl. 1st thousand. 1909. 3 p. l., 278 p. incl. illus., tables. pl., fold. map.

Conservation by sanitation. Air and water disposal of waste. (including a laboratory guide for sanitary engineers.) New York, John Wiley and sons. 1911. 12 and 305 p.
RICHARDSON, JOSEPH GIBBONS, 1836–1886.
A handbook of medical microscopy . . . Philadelphia, J. B. Lippincott and co., 1871. viii, 9–333 p. illus.
RICKARDS, BURT RANSOM. See Biological studies by the pupils of William Thompson Sedgwick.
RICKETTS, HOWARD TAYLOR, 1871–1910.
Contributions to medical science by Howard Taylor Ricketts, 1870–1910; published as a tribute to his memory by his colleagues under the auspices of the Chicago patho-

logical society. Chicago, Ill., The University of Chicago press 1911 ix, 497 p. front. (port.) illus., plates (1 col.) ["Several articles by collaborators and pupils of Dr. Ricketts have been included because they were inspired by him and were in course of preparation before his death."—Introductory note.]

Infection, immunity and serum therapy in relation to the infectious diseases which attack man; with considerations of the allied subjects of agglutination, precipitation, hemolysis, etc. . . . Chicago, American medical association press, 1906. x, 600 p. illus. [Also, 1908]

Infection, immunity and serum therapy in relation to the infectious diseases of man. 2d ed., rev. and enl. by the author and by Geo. F. Dick . . . with preface by Ludvig Hektoen, M.D. Chicago, American medical association press, 1913. xv, 785 p. illus.

Riesman, David. (comp.) See Carter, William S. (comp.)

Riesman, David. See Hektoen, Ludvig, (ed.)

[Riley, William Durnell]
Locusts and grasshoppers. The beginning and the end of the febrile or eruptive diseases in living things. [Philadelphia, 1872] cover-title, 8 p.

Ritchie, James. See Muir, Robert.

Ritchie, John W.
Primer on sanitation; being a simple work on disease germs and how to fight them. Yonkers-on-Hudson, 1910. 200 p.

Roberts, George Fairclough, 1886–
Nurses manual of bacteriology and aseptic technique . . . Salt Lake City, Utah, Western printing co. [⁰1915] 1 p. l., 3–21 numb. l.

Roberts, Jay G.
Manual of bacteriology and pathology for nurses . . . Philadelphia and London, W. B. Saunders company, 1912. 2 p. l., 3–206 p. VIII pl (3 col.) [Also, 1914]

Rogers, Anne F. See Biological studies by the pupils of William Thompson Sedgwick.

Rosenau, M. J.
Disinfection and disinfectants. A practical guide for sanitarians, health and quarantine officers. Philadelphia, P. Blakiston's son and co., 1902. xii, 17–353 p. incl. illus. . . . pl., diagr.

Rostoski, Otto.
Manual of serum diagnosis . . . Authorized translation by Dr. Charles Bolduan. 1st ed. 1st thousand. New York, J. Wiley and sons; London, Chapman and Hall, limited, 1904. vi, 86 p., diagrs.

Russell, Harry Luman, 1866–
Agricultural bacteriology. A brief outline of bacteria in their relation to agriculture. Part II. Madison, [State journal printing co.], 1903. 38 p. [Bound with 6th edition of Outlines of Dairy Bacteriology.]

Agricultural bacteriology, by H. L. Russell . . . and E. G. Hastings . . . Madison, Wis., H. L. Russell, 1909. vi, 241 p. illus., diagrs.

Agricultural bacteriology for students in general agriculture, by H. L. Russell . . . and E. G. Hastings . . . Madison, Wis., H. L. Russell, 1915. vi, 304 p. illus., diagrs.

Bacterial life of milk . . . Address delivered before the Ohio state dairymen's association in the University chapel on February 6, 1901 . . . Columbus, The University [1901?] cover-title, 15, [1] p. illus.

Experimental dairy bacteriology, by H. L. Russell . . . and E. G. Hastings . . . Boston, New York [etc.] Ginn and company [⁰1909] viii, 147 p. illus., chart (in pocket)

Outlines of dairy bacteriology ... Madison, Wis., The author, 1894.   vi p., 1 l., 186 p.

Outlines of dairy bacteriology, a concise manual for the use of students in dairying ... 2d ed. thoroughly rev. ... Madison, Wis., The author, 1896.   192 p.   illus., diagrs.

———— 4th ed., thoroughly revised. Madison, Wis., H. L. Russell, 1899.   vi p., 1 l., 190 p.   illus.

———— 6th ed., thoroughly rev.   1905.   iv p., 1 l., 199 p.   illus.

———— 9th ed., wholly rewritten.   1910.   214 p.   illus.

———— 10th ed.   1914.   223 p.   illus.

RUSSELL, HARRY LUMAN.   See Turneaure, F. E.

SALISBURY, JAMES HENRY, 1823–1905.

Microscopic examinations of blood; and vegetations found in variola, vaccina, and typhoid fever. New York, Moorhead, Bond and co., printers, 1868.   iv p., 1 l., [7]–65, [3] p. incl. illus., 3 pl.

SALOMONSEN, CARL JULIUS, 1847–1924.

Bacteriological technology for physicians with seventy-two figures in the text by Dr. C. J. Salomonsen. Authorized translation from the 2d rev. Danish ed., by William Trelease. New York, W. Wood and company, 1890.   2 p. l., 162 p.   illus.   [See also Wood's Medical and Surgical Monographs 4: 437–597, 1889]

SATTERTHWAITE, THOMAS EDWARD, 1843–

An introduction to practical bacteriology ... Detroit, Mich., G. S. Davis, 1887.   5 p. l., 85 p. front., illus.

SAYRE, LUCIUS ELMER, 1847–1925.

A manual of organic materia medica and pharmacognosy; and introduction to the study of the vegetable kingdom and the vegetable and animal drugs ... 2d ed., rev., with histology and microtechnique by William C. Stevens ... With 374 illustrations Philadelphia, P. Blakiston's sons and co., 1899.   xvii, 17–684 p. incl. illus., plates, map.

SCHAMBERG, JAY F.   See Welch, William Miller.

SCHMITZ, J[OHN] P[ETER]

The microbe-producing-disease theory inconsistent with the laws of nature; how diseases are produced, a new physiological law promulgated by Prof. J. P. Schmitz. San Francisco, California, The author, 1901.   [5]–50 p.

SCHNEIDER, ALBERT, 1863–1928.

Bacteriological methods in food and drug laboratories, with an introduction to micro-analytic methods ... 87 illustrations and 6 full page plates. Philadelphia, P. Blakiston's son and co. [c1915] viii, 288 p.   illus. (part col.) VI pl.

Pharmaceutical bacteriology, with special reference to disinfection and sterilization ... with 86 illustrations. Philadelphia, P. Blakiston's son and company, 1912.   viii, 238 p. illus.

SCHORER, EDWIN HENRY, 1879–

Vaccine and serum therapy, including also a study of infections, theories of immunity, opsonins and opsonic index ... St. Louis, C. V. Mosby co., 1909.   131 p.   illus., diagrs. [Also, 1912]

Vaccine and serum therapy, including also a study of infections, theories of immunity, specific diagnosis and chemotherapy ... 2d rev. ed. St. Louis, C. V. Mosby company, 1913.   2 p. l., ix–xv, 300 p. illus., col. pl., diagrs.

Seogwick, William Thompson, 1855–1921.

. Lectures delivered to the employes of the Baltimore and Ohio railroad company. III. on fermentation. Baltimore, printed and lithographed by Isaac Friedenwald, 1882. 73 p. illus.

———

Principles of sanitary science and the public health with special reference to the causation and prevention of infectious diseases ... New York, The Macmillan company; London, Macmillan and co., ltd., 1902. xix, 368 p. front. Also, 1903.

Sedgwick, William Thompson. See Whipple, George C.

Seidensticker, Oswald, (ed.) See Cohn, Ferdinand Julius.

Senn, Nicholas, 1844–1908.

Bactériologie chirurgicale, tr. by A. Broca ... Paris, G. Steinhall, 1890. 318 p.

———

The relation of microörganisms to injuries and surgical diseases by N. Senn. M.D., Ph.D., Milwaukee, Wisconsin. 2 vol. mms. [Published under title *Surgical bacteriology* in 1889. Another copy on 344 typewritten sheets.]

———

Surgical bacteriology ... Philadelphia, Lea brothers and co., 1889. 4 p. l., [17]–270 p. XIII (*i.e.* 12) pl. (part col.)

———

——— 2d ed. thoroughly rev. 1891. viii, [17]–271 p. illus., XIII (*i.e.* 12) pl. (part col.) [Copy in Crerar Library with numerous mms. notes and clippings by author.]

Shattock, Samuel George.

An atlas of the bacteria pathogenic in man, with descriptions of their morphology and modes of microscopic examination ... With an introductory chapter on bacteriology: its practical value to the general practitioner. By W. Wayne Babcock, M.D. ... New York, E. B. Treat and co., 1899. 82 p. incl. illus., 16 col. pl.

Shecut, John Linnaeus Edward Whitridge, 1770–1836.

Shecut's medical and philosophical essays. Containing: 1st. Topographical, historical and other sketches of the city of Charleston, from its first settlement to the present period. 2d. An essay on the prevailing fever of 1817. 2d ed., with improvements. 3d. An essay on contagions and infections. (2d improved ed.) And, 4th. An essay on the principles and properties of the electric fluid. The whole of which are designed as illustrative of the domestic origin of the yellow fever of Charleston; and, as conducing to the formation of a medical history of the state of South-Carolina ... Charleston, Printed for the author, by A. E. Miller, 1819. viii, 260, [2] p. pl.

Shepard, Hiram Hur, 1868–

Bacteria in foods; a treatise on the conservation of health; or the scientific preparation of pure food ... Chicago, A. Flanagan company, [°1911] 32 p.

Siears, Frank Bowker.

Elements of bacteriology. Being especially designed for the use of students as an introduction to the study of microorganisms. 1st ed. Cambridge, Mass., the Co-operative press, 1897. 72 p.

Simon, Charles Edmund, 1866–

Infection and immunity, a text-book of immunology and serology for students and practitioners ... 3d ed., rev. and enl. 111 Philadelphia and New York, Lea and Febiger, 1915. x, [17]–351 p. illus., XII pl. (part col.) [Previous editions published under title: An introduction to the study of infection and immunity.]

———

An introduction to the study of infection and immunity, including chapters on serum therapy, vaccine therapy, chemotherapy and serum diagnosis for students and practitioners ... Philadelphia and New York, Lea and Febiger, 1912. x, [17]–201 p. illus., XI pl. (partly col.)

———— 2d ed., rev. and enl., 1913. x, [17]–325 p. illus., XI pl. (part col.) diagrs.

A manual of clinical diagnosis by means of microscopic and chemical methods, for students, hospital physicians, and practitioners...With 132 illustrations on wood and 10 colored plates. Philadelphia and New York, Lea brothers and co., 1896. 2 p. l., [vii]–xix, [17]–504 p. illus. (part col.) X col. pl., diagrs.

2d ed., rev. and enl. With 133 illustrations on wood and 14 colored plates., 1897. xx, 17–563 p. illus., XIV col. pl., diagrs.

———— 3d ed., thoroughly rev. Illustrated with 136 engravings and 18 plates in colors, 1900. xxiv, 17–558 p. illus., XVIII col. pl.

———— 4th ed., thoroughly rev. Illustrated with 139 engravings and 19 plates in colors, 1902. xxiv, 17–599 p. illus., XIX col. pl.

———— 5th ed., thoroughly rev. and enl. Illustrated with 150 engravings and 22 plates in colors, 1904. xxiv, 17–695 p. illus., 22 col. pl.

———— 6th ed., thoroughly rev. Illustrated with 177 engravings and 24 plates in colors, [c1907]. xix, [17]–682 p. illus., XXIV pl. (23 col.)

———— 7th ed. enl. and thoroughly rev. Illustrated with 168 engravings and 25 plates. Philadelphia and New York, Lea and Febiger, 1911. xviii, [17]–778 p. illus. XXV pl. (23 col.)

———— 8th ed. enl. and thoroughly rev. Illustrated with 185 engravings and 25 plates, 1914. xviii, [17]–809 p. illus., XXV pl. (23 col., 1 fold.)

SIMONDS, JAMES PERSONS, 1878–
. . . Studies in *Bacillus welchii*, with special reference to classification and to its relation to diarrhea. New York, The Rockefeller Institute for Medical Research, 1915. 130 p. incl. tables. [Monographs of the Rockefeller Institute for Medical Research. No. 5].

SLADE, DANIEL DENISON, 1823–1896.
. . . Diphtheria; its nature and treatment, with an account of the history of its prevalence in various countries. Being the dissertation to which the Fiske fund prize was awarded July 11, 1860. Philadelphia, Blanchard and Lea, 1861. 2 p. l., [17]–85 p. illus.

———— Being a 2d and rev. ed. of an essay to which was awarded the Fiske fund prize of 1860. 1864. 2 p. l., [13]–166 p. illus.

SMITH, ALLEN JOHN, 1863–
Lessons and laboratory exercises in bacteriology; an outline of technical methods introductory to the systematic study and identification of bacteria, arranged, for the use of students . . . Philadelphia, P. Blakiston's son and co., 1902. 1 p. l., x, 9–298 p. illus., diagrs.

SMITH, ALLEN J[OHN]. See Kolmer, John Albert.

SMITH, ERWIN FRINK, 1854–1927.
Bacteria in relation to plant diseases . . . Washington, D. C., Carnegie institution of Washington, 1905–14. 3v. front., illus., plates (part col.) (*On verso of t.-p.:* Carnegie institution of Washington. Publication no. 27)

SMITH, JOHN HALL.
Lectures delivered by John Hall Smith, M.D., to the Massachusetts chiropody association (incorporated) 1910–1911. [Boston, J. R. Ruiter and co., printers, c1912]

127 p. *Partial contents:* 6. Bacteriology. 7. Suppuration; abscesses; toxins, blood poison; sapremia-toxemia; thrombosis—embolism. 8. Diseases of the foot.]

Society of American Bacteriologists.

—— Constitution and list of members. [Includes: History of organization. The Society, H. W. Conn, secretary and treasurer.] 1900. v, 1–5 p.

———— Constitution and list of members. [Chicago, The Society, Edwin O. Jordan, secretary and treasurer.] 1902. 15 p.

———— ———— [The Society, Frederic P. Gorham, secretary and treasurer.] 1903. 13 p.

———— Officers of the Society, constitution and list of members. [The Society, Norman MacL. Harris, secretary and treasurer.] 1908. 16 p.

———— ———— [The Society, Charles E. Marshall, secretary and treasurer.] 1912. 18 p.

———— ———— [The Society, A. Parker Hitchens, secretary and treasurer.] 1913. 23 p.

———— ———— [The Society, A. Parker Hitchens, secretary and treasurer.] 1914. 23 p.

———— ———— [The Society, A. Parker Hitchens, secretary and treasurer.] 1915. 27 p.

State Board of Health of Massachusetts.

A brief history of its organization and its work, 1869–1912. Boston, Wright and Potter printing co., 1912. 70 p.

Sternberg, George Miller, 1838–1915.

Disinfection and individual prophylaxis against infectious diseases. . . . Concord, N. H., Republican press association, 1886. 40 p. [American public health association. Lomb prize essay. (no. 3)]

———— . . . Disinfection and individual prophylaxis against infectious diseases. (Revised in December, 1899, by the author.) . . . Columbus, Ohio, The Berlin printing co., 1900. 45 p.

———— Immunity, protective inoculations in infectious diseases, and serum-therapy . . . New York, W. Wood and company, 1895. vi p., 2 l., 3–325 p. (*On cover:* Medical practitioner's library)

———— Infection and immunity, with special reference to the prevention of *infectious diseases* . . . New York and London, G. P. Putnam's sons, 1903. 1 p. l., xi, 293 p. *illus.* (*Half-title:* The science series . . . [12])

———— Malaria and malarial diseases . . . New York, W. Wood and company, 1884. vii, 329 p. diagrs. (*On cover:* Wood's library of standard medical authors)

———— A manual of bacteriology . . . Illustrated by heliotype and chromo-lithographic plates and two hundred and sixty-eight engravings. New York, W. Wood and company, 1892. xii, 886 p. illus. (part col.) VIII col. pl. [Also, 1893]

———— Photo-micrographs and how *to make* them . . . Boston, J. R. Osgood and company, 1883. xv, [17]–204 p. XX pl. (incl. front.) [Also, 1884]

———— Sanitary lessons of the war, and other papers . . . Wash[ington] D. C., Press of B. S. Adams, 1912. 2 p. l., 91 p.

A text-book of bacteriology ... Illustrated by heliotype and chromo-lithographic plates and two hundred engravings. 2d rev. ed. New York, W. Wood and company, 1901. 3 p. l., [v]-xi, 708 p. illus., IX pl. (part col.)

STERNBERG, G[EORGE] M[ILLER]. See American public health association, 1886.

———— See Magnin, Antoine.

STEVENS, WILLIAM C. See Sayre, Lucius Elmer.

STILES, PERCY GOLDTHWAIT. See Biological studies by the pupils of William Thompson Sedgwick.

STITT, EDWARD RHODES, 1867–

The diagnostics and treatment of tropical diseases ... with 86 illustrations. Philadelphia, P. Blakiston's son and co. [°1914] xi p., 1 l., 421 p. illus., diagrs.

Practical bacteriology, blood work and animal parasitology, including bacteriological keys, zoological tables and explanatory clinical notes ... with 86 illustrations. Philadelphia, P. Blakiston's son and co., 1909. xi, 294 p. illus., IV pl.

———— 2d ed., rev. and enl., with 91 illustrations, 1910. xiii, 345 p. illus., plates. [Also, 1911]

———— 3d ed., rev. and enl., with 4 plates and 106 other illustrations containing 513 figures, 1913. xv, 408 p. illus., IV pl. [Also, 1914]

STONEY, EMILY MARJORY ARMSTRONG.

Bacteriology and surgical technique for nurses ... Philadelphia, W. B. Saunders and company, 1900. 190 p. illus., 4 pl.

———— 2d ed., thoroughly rev. and enl. by Frederic Richardson Griffith ... Philadelphia—New York [etc.] W. B. Saunders and company, 1905. 2 p. l., 278 p. illus., 4 pl.

———— 3d ed., thoroughly rev. and enl. by Frederic Richardson Griffith ... Philadelphia and London, W. B. Saunders company, 1910. 2 p. l., 311 p. illus., plates.

THOMAS, BENJAMIN ABRAHAM, 1878–

Applied immunology; the practical application of sera and bacterins prophylactically, diagnostically and therapeutically; with an appendix on serum treatment of hemorrhage, organotherapy and chemotherapy by B. A. Thomas ... and R. H. Ivy ... 5 colored inserts and 68 illustrations in text. Philadelphia and London, J. B. Lippincott company [°1915] xv, 359 p. col. front., illus., plates (part col., 1 fold.) diagrs. (part fold.)

TRELEASE, WILLIAM, (tr.) See Salomonsen, Carl Julius.

TROUESSART, ÉDOUARD LOUIS, 1842–

Antiseptic therapeutics. By Dr. E. Trouessart ... Tr. by E. P. Hurd ... Detroit, Mich., G. S. Davis, 1893. 2 v.

TURNEAURE, FREDERICK EUGENE, 1866–

Public water-supplies. Requirements, resources, and the construction of works. By F. E. Turneaure, C. E., and H. L. Russell ... With a chapter on pumping-machinery, by D. W. Mead ... 1st ed. 2d thousand. New York, J. Wiley and sons; [etc., etc.] 1903. xiv, 746 p. incl. illus., plates, diagrs. [Also, 1906]

———— 2d ed. Rev. and enl. 1st thousand. 1908. xv, 808 p. incl. illus., plates, tables, diagrs.

TURNEAURE, FREDERICK EUGENE. See American school of correspondence.

TUTTLE, ALBERT HENRY, 1844–

An introduction to the study of the *Bacteria* . . . [Charlottesville, Va.] G. W. Olivier, 1893. vii, [9]–488 p.

U. S. *Library of Congress. Classification division.*

Classification. Class QR: Bacteriology (including yeasts, moulds, and pathogenic protozoa) [Washington, Govt. print. off., 1905] 1 p. l., p. 139–140.

VAUGHAN, GEORGE TULLY, 1859–

. . . The principles and practice of surgery, designed for students and practitioners . . . Philadelphia and London, J. B. Lippincott company, 1903. xiii, 569 p. illus., plates. (Lippincott's new medical series . . .)

VAUGHAN, J. WALTER. See Vaughan, Victor Clarence.

VAUGHAN, VICTOR CLARENCE, 1851–1929.

Cellular toxins; or, The chemical factors in the causation of disease, by Victor C. Vaughan . . . and Frederick G. Novy . . . 4th ed., rev. and enl. Philadelphia and New York, Lea brothers and company, 1902. viii, 17–495 p. illus., pl., tab.

————

Infection and immunity. Chicago, American medical association. 1915. 238 p.

————

Ptomaïnes and leucomaïnes, or, The putrefactive and physiological alkaloids. By Victor C. Vaughan . . . and Frederick G. Novy . . . Philadelphia, Lea brothers and co., 1888. viii, [13]–316 p. fold. tab.

————

Ptomaïnes, leucomaïnes, and bacterial proteids: or, The chemical factors in the causation of disease. . . . 2d ed., rev. and enl. 1891. x, [13]–391 p. fold. tab.

————

Ptomaïnes, leucomaïnes, toxins and antitoxins: or, The chemical factors in the causation of disease. 3d ed., rev. and enl. 1896. xii, [13]–604 p. fold. tab.

————

Protein split products in relation to immunity and disease, by Victor C. Vaughan . . . Victor C. Vaughan, jr. . . . and J. Walter Vaughan . . . Philadelphia and New York, Lea and Febiger, 1913. xii, [17]–476 p. incl. illus., tables, diagrs.

VAUGHAN, VICTOR CLARENCE. See American public health association, 1886.

VAUGHAN, VICTOR C., JR. See Vaughan, Victor Clarence.

VENABLE, WILLIAM MAYO, 1871–

Methods and devices for bacterial treatment of sewage . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1908. vii, 236 p., incl. illus., tables, diagrs. 3 pl. (1 fold.)

VOGEL, KARL MAX. See Ball, Michael Valentine.

WADSWORTH, AUGUSTUS. See Biological studies by the pupils of William Thompson Sedgwick.

WALLGREEN, ANDREW BENNETT.

Histology and bacteriology in abstract . . . Pittsburgh, Medical abstract co , 1906. 207 p.

WASSERMANN, AUGUST VON, 1866–1925.

Immune sera; haemolysins, cytotoxins, and precipitins, by Prof. A. Wassermann . . . authorized translation by Charles Bolduan, M.D. 1st ed., 1st thousand. New York, J. Wiley and sons; London, Chapman and Hall, limited, 1904. ix, 77 p. illus

WEAVER, GEORGE HOWITT. See Lehmann, Karl Bernhard.

WEBSTER, RALPH WALDO, 1873–

Diagnostic methods, chemical, bacteriological and microscopical; a text-book for students and practitioners, by Ralph W. Webster . . . with 37 colored plates and 164 other illustrations. Philadelphia, P. Blakiston's son and co., 1909. xxxiv, 641 p illus. (part col.) XXXVII col. pl.

———— . . . 2d ed., rev. and enl., with 37 colored plates and 164 other illustrations. 1912. xxxv, 682 p. illus., XXXVII pl. (35 col.)

———— . . . 3d ed., rev. and enl., with 37 colored plates, and 164 other illustrations. 1913. xxxvii, 692 p. illus., XXXVII pl. (35 col.)

———— . . . 4th ed., rev. and enl., with 37 colored plates and 171 other illustrations. Philadelphia, P. Blakiston's son and co. [c1914] xxxvi, 738 p. illus. (part col.) XXXVII pl. (part col.)

WELCH, WILLIAM HENRY.

Lectures on bacteriology, serum therapy and immunity (delivered before the second year students, Oct.–Dec., 1907) Baltimore, 1908.

WELCH, WILLIAM MILLER, 1837–

Acute contagious diseases. By William M. Welch . . . and Jay F. Schamberg . . . Illustrated with 109 engravings and 61 full-page plates. Philadelphia and New York, Lea brothers and co., 1905. 1 p. l., [v]–viii, [17]–781 p. illus., LXI pl.

WELLS, H. GIDEON.

Chemical pathology . . . being a discussion of general pathology from the standpoint of the chemical processes involved. Philadelphia, W. B. Saunders company. 1907. 549 p.

WENDT, EDMUND CHARLES.

A treatise on Asiatic cholera . . . ed. and prepared by Edmund Charles Wendt in association with John C. Peters, Ely McClellan, John B. Hamilton, and Geo. M. Sternberg. New York, William Wood and co., 1885. illus. with maps and drawings. 403 p.

[Western soil bacteria company, *San Francisco*]

County homes for busy little people. [San Francisco, Printed by Taylor and Taylor, c1915] [20] p. illus.

WESTON. PAUL GARFIELD. See Ball, Michael Valentine.

WHIPPLE, GEORGE CHANDLER, 1866–1924.

The microscopy of drinking-water . . . 1st ed., 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1899. xii, 300 p., 19 l. illus., XIX pl., diagrs.

———— 2d ed., rev. 1st thousand, 1905. xiii, 323 p. illus., XIX pl., diagrs. [Also 1906, 1908, 1910]

———— 3d ed., rewritten and enl. with colored plates. 1st thousand. New York, John Wiley and sons, inc.; [etc., etc.] 1914. xxi, 409 p. incl. front., illus., tables, diagrs. XIX (*i.e.* 20) col. pl.

Report on the quality of the water supply of the city of Cleveland, O. . . . Cleveland, Ohio, The Britton printing co., 1905. 201 p.

Typhoid fever; its causation, transmission and prevention . . . with an introductory essay by William T. Sedgwick . . . 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1908. xxxvi, 407 p. incl. front., illus., tables. map, tables diagrs. (part fold.)

The value of pure water. 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1907. viii, 84 p. incl. tables, diagr. [Appeared originally in a collection of scientific papers entitled *Biological studies by the pupils of William Thompson Sedgwick.*]

WHIPPLE, GEORGE CHANDLER. See Biological studies by the pupils of William Thompson Sedgwick.

WILLIAMS, ANNA WESSELS. See Park, William Hallock.
WILLIAMS, HERBERT UPHAM, 1866–
  A manual of bacteriology . . . With seventy-eight illustrations. Philadelphia, P. Blakiston's son and co., 1898. x, 9–263 p. illus.

———— with ninety-nine illustrations. 3d ed., rev. and enl. 1903. xv, 11–351 p. illus., pl.

———— rev. by B. Meade Bolton . . . with 108 illustrations. 4th ed., rev. and enl. 1905. xix, 357 p. illus., diagrs. [Also, 1906]

———— rev. by B. Meade Bolton . . . with 113 illustrations. 5th ed., rev. and enl., 1908. xiii, 466 p. illus., diagrs. [Also, 1909]

WILSON, JOSEPH MILLER, 1838–1902.
  Drainage for health; or, Easy lessons in sanitary science. Philadelphia, P. Blakiston, 1881 [1880] 1 p. l., 68 p. front., illus.

WILSON, SAMUEL JEROME.
  Microbes and health, by Samuel J. Wilson . . . [Lansing? Mich.] Pub. by the author, 1901. 2 p. l., [vii]–viii, 230 p.

WINSLOW, ANNE ROGERS. See Winslow, Charles-Edward Amory.

WINSLOW, CHARLES-EDWARD AMORY, 1877–
  Elements of applied microscopy. A text-book for beginners . . . 1st ed. 1st thousand. New York, J. Wiley and sons; London, Chapman and Hall, limited, 1905. xii, 183 p. illus., diagrs.

  The systematic relationships of the Coccaceae, with a discussion of the principles of bacterial classification, by Charles-Edward Amory Winslow . . . and Anne Rogers Winslow. 1st ed. 1st thousand. New York, J. Wiley and sons; [etc., etc.] 1908. viii p., 1 l., 300 p. front. (col. chart) diagrs.

WINSLOW, CHARLES-EDWARD AMORY. See Biological studies by the pupils of William Thompson Sedgwick.
———— See Prescott, Samuel Cate.

WINSLOW, KENELM.
  Germ diseases. Part II of Vol. I of the Home Medical Library. New York, Review of reviews co., 1907. pp. 191–266.

WOLL, FRITZ WILHELM, (ed.) See Grotenfelt, Gösta.

Women's educational and industrial union, Boston. Appointment Bureau.
  Bacteriological work as a vocation for women. Prepared by the Appointment bureau, Women's educational and industrial union . . . Boston, Mass. [¹1911] 8 p. (On cover: Vocation series. Bulletin no. 9. July, 1911)

WOOD, EUGENE HALSEY.
  The quest of the germ: with observations thereanent . . . Milwaukee, Wis., The author, 1906. 2 p. l., ii–iii, ii, [9]–229 p. front., illus.

WOOD, FRANCIS CARTER. See Delafield, Francis.

WOODMAN, ALPHEUS GRANT, 1873–
  Air, water, and food from a sanitary standpoint, by Alpheus G. Woodman . . . and John F. Norton . . . 4th ed., rev. and rewritten. Total issue, five thousand. New York, J. Wiley and sons, inc.; [etc., etc.] 1914. v, 248 p. incl. illus., tables, diagrs. fold. map.

WOODMAN, ALPHEUS GRANT. See Richards, Ellen H.

WRIGHT, JAMES HOMER. See Mallory, Frank Burr.

WRIGHT, W. H. See Hastings, Edwin George.

WYTHE, JOSEPH HENRY, 1822–1901.
  The microscopist; or, A complete manual on the use of the microscope . . . 2d ed.,

impr. and enl. . . . By Joseph H. Wythes [!] M.D. Philadelphia, Lindsay and Blakiston; London, Trubner and co., 1853. viii p., 1 l., [13]–212 p. illus., 2 pl. (incl. front.) diagrs. [1st ed. appeared in 1851]

The microscopist: a manual of microscopy and compendium of the microscopic sciences . . . 3d ed. Rewritten and greatly enl. . . . Philadelphia, Lindsay and Blakiston, 1877. xi, [17]–259, [1] p. incl. front., illus. XXVII col. pl.

———— 4th ed.; greatly enl. . . . 1880. xii, [17]–434 p. incl. front., illus. XXVII col. pl.

ZAPFFE, FREDERICK CARL, 1873–
. . . Bacteriology. A manual for students and practitioners . . . illustrated with one hundred and forty-six engravings and seven colored plates. Philadelphia and New York, Lea brothers and co. [1903] 8, 17–350 p. illus., 7 col. pl. (incl. front.) (Lea's series of pocket text-books.)

ZIEGLER, GEORGE JACOB, 1821–
The basic pathology and specific treatment of diphtheria, typhoid, zymotic septic, scorbutic, and putrescent diseases generally . . . Philadelphia, G. J. Ziegler, 1884. 225 p.

ZINSSER, HANS. See Hiss, Philip Hanson.

SECTION II

CHRONOLOGICAL LIST OF AUTHORS

1794
Condict, Lewis

1798
A physical enquiry . . . .

1799
Caldwell, Charles

1801
Caldwell, Charles

1815
Gallup, Joseph A.

1819
Shecut, John Linnaeus Edward Whitridge

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Hosack, David

1823
Jameson, Horatio Gates

1849
Mitchell, John Kearsley

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Wythe, Joseph H[enry]

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Harris, Elisha

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Mitchell, John Kearsley

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Slade, Daniel Denison

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Slade, Daniel Denison

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Salisbury, James Henry

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Richardson, Joseph Gibbons
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1874
Dalton, John C[all]

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Logan, Cornelius Ambrose

1894

Abbott, Alexander Crever
Coplin, William Michael Late
Grotenfelt, Gösta
Hare, Hobart Amory
MacFarlane, Andrew
Novy, Frederick George
Orcutt, I. H.
Reeves, James Edmund
Russell, Harry Luman

1895

Abbott, Alexander Crever
Buchanan, Charles Milton
Carter, William S. (comp.)
[Ernst, Harold Clarence]
Frothingham, Langdon
Grotenfelt, Gösta
Hare, Hobart Amory
Herrick, James B.
Leffmann, Henry
Radam, William
Sternberg, George Miller

1896

Delafield, Francis and Prudden, T. Mitchell
McFarland, Joseph
Mason, William Pitt
Russell, Harry Luman
Simon, Charles Edmund
Vaughan, Victor Clarence and Novy, Frederick G.

1897

Abbott, Alexander Crever
Ball, Michael Valentine
Conn, Herbert William
Coplin, William Michael Late
Fuertes, James Hillhouse
Gorham, Frederic Poole
Hare, Hobart Amory
Mallory, Frank Burr and Wright, James Homer
Mulford, H. K. Company
Siears, Frank Bowker
Simon, Charles Edmund

1898

American public health association
Hare, Hobart Amory
Hayes, Matthew Horace
Moore, Veranus Alva
Pfingst, Adolph O. and Cashin, John E.
Potter, Theodore
Williams, Herbert Upham

1899

Abbott, Alexander Crever
Bowhill, Thomas
Duckwall, Edward Wiley
Hueppe, Ferdinand Adolph Theophil. Tr. by Dr. E. O. Jordan
Leffmann, Henry
Lentz, Chas., and Sons
Mason, William Pitt
Novy, Frederick George
Osburn, William
Park, William Hallock
Russell, Harry Luman
Sayre, Lucius Elmer
Shattock, Samuel George
Whipple, George Chandler

1900

Ball, Michael Valentine
Coplin, William Michael Late
Ernst, Harold C.
Hare, Hobart Amory
Levy, Ernst and Klemperer, Felix. Tr. by Augustus A. Eshner
Marchiafava, Ettore; Bignami, Amico; Flexner, Simon; and Opie, Eugene L.
McFarland, Joseph
Moore, Veranus Alva
Richards, Ellen Henrietta (Swallow) "Mrs. R. H. Richards," and Woodman, Alpheus G.
Simon, Charles Edmund
Society of American Bacteriologists
Sternberg, George Miller
Stoney, Emily Marjory Armstrong

1901

Abbott, Alexander Crever
Chapin, Charles V.
Chester, Frederick Dixon
Conn, Herbert William
Delafield, Francis and Prudden, T. Mitchell
Frost, William Dodge
Gorham, Frederic Poole
Hatfield, Marcus Patten
Hektoen, Ludvig and Riesman, David (ed.)
Leffman, Henry
Lehmann, Karl Bernhard
Mallory, Frank Burr and Wright, James Homer
Mason, William Pitt
Palisade manufacturing company
Price, George M.
Russell, Harry Luman

Schmitz, J[ohn] P[eter]
Sternberg, George Miller
Wilson. Samuel Jerome

1902

Abbott, Alexander Crever
Chapin, Henry Dwight
Conn, Herbert William
Councilman, William Thomas and Mallory, F. B.
Frost, William Dodge
Hare. Hobart Amory
Harvard University, Medical School
Mason, William Pitt
Moore, Veranus Alva
Rosenau, M. J.
Sedgwick, William Thompson
Simon, Charles Edmund
Smith, Allen John
Society of American Bacteriologists
Vaughan, Victor Clarence and Novy, Frederick G.

1903

Archinard, Paul Émile
Barnes, Carl Lewis
Belcher, Sarah Drowne, "Mrs. E. R. Hardy."
Chicago. University.
Conn, Herbert William
Cross, M. I.
Ernst, Harold Clarence
Frost, William Dodge
Jousset, Pierre. *Tr.* by Horace P. Holmes
Leffmann, Henry
McFarland, Joseph
Muir, Robert and Ritchie, James
Russell, H. L.
Sternberg, George Miller
Society of American Bacteriologists
Turneaure, Frederick Eugene and Russell, H. L.
Vaughan, George Tully
Williams, Herbert Upham
Zapffe, Frederick Carl

1904

Anatomy, physiology, pathology ....
Ball, Michael Valentine
Behring, Emil Adolph von. *Tr.* by Charles Bolduan
Chapin, Henry Dwight
Delafield, Francis and Prudden, T. Mitchell
Flexner, Simon and Holt, L. Emmet

Hare, Hobart Amory
Lenhartz, Hermann. *Tr.* by Henry T. Brooks
Mallory, Frank Burr and Wright, James Homer
Marshall, Charles E.
Prescott, Samuel Cate and Winslow, Charles-Edward Amory
Reid, Mary Eliza
Richards, *Mrs.* Ellen Henrietta (Swallow) and Woodman, Alpheus G.
Rostoski, Otto. *Tr.* by Charles Bolduan
Simon, Charles Edmund
Wassermann, August von. *Tr.* by Charles Bolduan

1905

Abbott, Alexander Crever
American public health association, *Laboratory section*
Coplin, William Michael Late
Duckwall, Edward Wiley
Hare, Hobart Amory
Heinemann, Paul Gustav
Levy, Ernest Coleman
Moore, Veranus Alva
Park, William Hallock
Russell, Harry Luman
Smith, Erwin Frink
Stoney, Emily Marjory Armstrong
U. S. *Library of Congress. Classification division*
Welch, William Miller and Schamberg, Jay F.
Whipple, George Chandler
Williams, Herbert Upham
Winslow, Charles-Edward Amory

1906

Biological studies by the pupils of William Thompson Sedgwick
Ehrlich, Paul. *Tr.* by Charles Bolduan
McFarland, Joseph
Mason, William Pitt
Moore, Veranus Alva
Ricketts, Howard Taylor
Wallgren, Andrew Bennett
Wood, Eugene Halsey

1907

Arrhenius, Svante August
Ball, Michael Valentine
Bolduan, Charles Frederick
Conn, Herbert William
Delafield, Francis and Prudden, T. Mitchell

Eimer and Amend
Hare, Hobart Amory
Herter, Christian Archibald
Jowett, Walter
Langfeld, Millard
Pitfield, Robert Lucas
Simon, Charles Edmund
Wells, H. Gideon
Whipple, George Chandler
Winslow, Kenelm

1908

American school of correspondence
Ball, Michael Valentine
Bolduan, Charles Frederick
Grotenfelt, Gösta
Jordan, Edwin Oakes
Lipman, Jacob Goodale
Mallory, Frank Burr and Wright, James Homer
Moore, Veranus Alva
Nuttall, George Henry Falkiner (ed.)
Palmer, W. C.
Park, William Hallock
Prescott, Samuel Cate and Winslow, Charles-Edward Amory
Society of American Bacteriologists
Turneaure, Frederick Eugene and Russell, H. L.
Venable, William Mayo
Welch, William H.
Whipple, George Chandler
Williams, Herbert Upham
Winslow, Charles-Edward Amory and Winslow, Anne Rogers

1909

Abbott, Alexander Crever
American school of correspondence
Bordet, Jules. Tr. by Frederick P. Gay.
Conn, Herbert William
Dieudonné, Adolph. Tr. by Charles Frederick Bolduan
Earp-Thomas farmogerm co.
Eliot, Charles William
Faught, Francis Ashley
Hare, Hobart Amory
Leffmann, Henry
Levy, Ernest Coleman
McCampbell, Eugene Franklin
McFarland, Joseph
McIsaac, Isabel
Richards, Mrs. Ellen Henrietta (Swallow) and Woodman, Alpheus G.
Russell, Harry Luman and Hastings, E. G.

Schorer, Edwin Henry
Stitt, Edward Rhodes
Webster, Ralph Waldo

1910

American public health association. Laboratory section
Chapin, Charles Value
Coplin, William Michael Late
Deason, J.
Doane, Rennie Wilbur
Ehrlich, Paul. Tr. by Charles Bolduan
Elliott, Sophronia Maria
Faught, Francis Ashley
Frost, William Dodge and McCampbell, Eugene Franklin
Herzog, Maximilian Joseph
Hiss, Philip Hanson and Zinsser, Hans
Jordan, Edwin Oakes
MacCaughey, Vaughan
Mason, William Pitt
Mears, James Ewing
Mexico. Ministerio de instrucción pública y bellas artes
Park, William Hallock and Williams, Anna W.
Prudden, Theophil Mitchell
Ritchie, John W.
Russell, Harry Luman and Hastings, E. G.
Stitt, Edward R.
Stoney, Emily Marjory Armstrong

1911

Bolduan, Charles Frederick
Buchanan, Robert Earle
Coplin, William Michael Late
Deason, J.
Delafield, Francis and Prudden, T. Mitchell
Doty, Alvah Hunt
Faught, Francis Ashley
Flexner, Simon
Frost, William Dodge
Harvard university. Medical school
Hastings, Edwin George; Hoffman, Conrad; and Wright, W. H.
Heinemann, Paul G.
Mallory, Frank Burr and Wright, James Homer
Marshall, Charles Edward (ed.)
Mulford, H. K., company
Richards, Ellen H.
Ricketts, Howard Taylor
Shepard, Hiram Hur
Simon, Charles Edmund

Women's educational and industrial union,
   Boston. Appointment bureau

1912

American public health association,
   Laboratory section
American school of home economics
Archinard, Paul Émile
Chapin, Charles Value
Citron, Julius Bernhard. *Tr.* by A. L.
   Garbat
Conn, Herbert William
Councilman, W. T.
Faught, Francis Ashley
Fox, Herbert
Frost, William Dodge and O'Shea, M. V.
Hare, Hobart Amory
Jordan, Edwin Oakes
Klopstock, Martin and Kowarsky, A.
McFarland, Joseph
McKail, David
Moore, Veranus Alva
Roberts, Jay G.
Schneider, Albert
Simon, Charles Edmund
Smith, John Hall
Society of American Bacteriologists
State Board of Health of Massachusetts
Sternberg, George Miller
Webster, Ralph Waldo

1913

Allen, Richard William
Ball, Michael Valentine
Bolduan, Charles Frederick and Grund,
   Marie
Buchanan, *Mrs.* Estelle Denis (Fogel) and
   Buchanan, Robert Earle
Burnham, Frederick W. E.
Councilman, William Thomas
Harvard University. *Medical school*
Heinemann, Paul Gustav
Hersey, Harvey
Miller, Frank William
Moore, Veranus Alva
Pitfield, Robert Lucas
Poulton, Edward Bagnall
Prescott, Samuel Cate and Winslow,
   Charles-Edward Amory
Putnam, John J.
Ricketts, Howard Taylor
Schorer, Edwin Henry
Simon, Charles Edmund
Society of American Bacteriologists
Stitt, Edward Rhodes

Vaughan, Victor Clarence; Vaughan, Vic-
   tor C., jr.; and Vaughan, Walter
Webster, Ralph Waldo

1914

A manual of biological therapeutics
Citron, Julius Bernhard. *Tr.* by A. L.
   Garbat
Delafield, Francis and Prudden, T. Mitchell
Hare, Hobart Amory
Hiss, Philip Hanson and Zinsser, Hans
Jordan, Edwin Oakes
McIsaac, Isabel
MacNeal, Ward J.
Mallory, Frank Burr
Moore, Veranus Alva and Fitch, Clifford
   P.
Osler, *Sir* William, *bart.*
Park, William Hallock and Williams, Anna
   W.
Peebles, Isaac Lockhart
Reed, Howard Sprague
Roberts, Jay G.
Russell, Harry Luman and Hastings, E. G.
Simon, Charles Edmund
Society of American Bacteriologists
Stitt, Edward Rhodes
Webster, Ralph Waldo
Whipple, George Chandler
Woodman, Alpheus Grant and Norton,
   John F.

1915

Abbott, Alexander Crever
Carey, Harry Wardwell
Conn, Herbert William
Faught, Francis Ashley
Janeway, Henry Harrington
Kolmer, John Albert
Lederle antitoxin laboratories. *Medical
   department*
Leffmann, Henry
McConnell, Guthrie
McFarland, Joseph
Mallory, Frank Burr and Wright, James
   Homer
Osborne, Oliver Thomas
Roberts, George Fairclough
Russell, Harry Luman and Hastings, E. G.
Schneider, Albert
Simon, Charles Edmund
Simonds, James Persons
Society of American Bacteriologists
Thomas, Benjamin Abraham
Vaughan, Victor Clarence
[Western soil bacteria company]

MICROBIOLOGICAL PROBLEMS IN THE PRESERVATION OF MEATS

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CONTENTS

Until some sixty years ago, man depended largely upon the perishable food supplies produced within a few hundred miles of his home. In general, the methods of preparation and the kinds of foodstuffs of animal origin had changed little since ancient times. With the rise of bacteriology, coupled with developments in the physical and chemical sciences, procedures have been elaborated for the control and study of meat foods. However, the pace of all historical development teaches us that the work in meat technology, as in other fields of food technology, has just begun.

For the preservation of meats, the following practical methods have been developed, some of them with an empirical background of great antiquity: partial drying and dehydration; curing with salt, nitrates, nitrites, and sugars; smoking, icing and refrigeration; culinary heating; packing in metal and glass containers. The maintenance of good standards of quality as well as of sanitation in the products often depends on adequate microbiological control; and, on occasion, specific microbiological problems are presented which depend, for an ultimate solution, upon fundamental research.

Practically every process employed in the meat industry hinges upon the reduction and control of microbiological activities. The proteins, carbohydrates and fats of meat foods are all vulnerable to the action of many kinds of microorganisms. There are numerous species of nonpathogenic bacteria, yeasts, and

molds which can discolor fresh and cured meats by oxidative processes; induce oxidative rancidity in fats, and discolor fats and oils with their pigments; ferment cured meats and canned meats with carbon dioxide production; and cause spoilage of unprotected proteins in a number of ways. For discussion of the many activities of microörganisms in meat foods and practical measures for control of microörganisms in meat plants, the reader is referred elsewhere (36).

The following sections illustrate a few of the problems arising from large-scale processing of meat foods which may be of general bacteriological interest.

1. ACTION OF CERTAIN BACTERIA ON MYOHEMOGLOBIN AND NITRIC-OXIDE DERIVATIVES FORMED IN CURED MEATS

The color of muscle tissue is red in the fresh, unheated state, but this color is not due to residual blood or hemoglobin. The pigment is myohemoglobin (or myoglobin), an integral part of the muscle tissue, which does not circulate in the blood stream. Hektoen, Robscheit-Robbins, and Whipple (27), by means of the precipitin reaction, have demonstrated the non-identity of myoglobin and hemoglobin. However, the general properties of these two pigments are sufficiently alike so that one may use the blood pigment for certain experiments in place of the muscle pigment without serious error in the conclusions drawn therefrom, according to Urbain and Jensen (103).

Unlike fresh meats, the cured meats retain their redness on cooking. Chiefly through the work of Haldane (25) and Hoagland (29), the stability of the cured meat pigment towards heat has been shown to be a property of nitric-oxide derivatives of the pigment resulting from the chemical interaction with nitrite either added directly in the curing salts or produced through bacterial reduction of nitrate. (These investigators assumed the pigment of meat to be identical with the blood pigment.) Successful curing of the meat eventually leads to the formation of nitric-oxide myohemoglobin, according to the reaction: NO + myohemoglobin ⇌ nitric-oxide myohemoglobin. The source of the nitric oxide is nitrite or more properly nitrous acid. Upon heating, the nitric-oxide myohemoglobin is converted to nitric-oxide hemochromogen, a denatured protein. The fact that this hemochromogen is red is one of the objects gained by the employment of nitrite and nitrate in the curing of meats. Any reaction which yields unusual colors in cured meats is undesirable as a matter of custom rather than of sanitary deficiency.

In the past, one of the most troublesome phenomena in the meat plant was the discoloration of cured meats, such as green rings in frankfurters, green boiled hams, and dried beef hams. The pink nitric-oxide pigments are sensitive to oxidations of microbial origin and become emerald green, gray, and brown in color when "oxidase"-forming bacteria act upon them. Reducing agents can often restore the pink color but the use of compounds, such as sulfites, is prohibited by law. The greening action of a few species of bacteria on blood agar media is so characteristic that considerable diagnostic importance has been attached to the phenomenon. The green pigment formed has been ascribed to a variety of

causes, among which are the following: formation of methemoglobin, Zinsser and Bayne-Jones (115), and Valentine (104); action of lactic acid, Ruediger (87) and Hagan (24); oxidation, McLeod and Gordon (71); reduction, Holman (31); xanthroproteic reaction, Barnard and Gowen (4); and an optical illusion, Boxer (7).

Jensen and Urbain (51) have shown that many species of bacteria possess mechanisms for discoloring blood pigments and their nitric-oxide derivatives. They found that the kinds of microörganisms responsible for the formation of green pigments in meats and blood-agar are those which oxidize hemoglobin, nitric-oxide hemoglobin, nitric-oxide hemochromogen, and hematin. In addition, bacteria producing hydrogen sulfide cause green discolorations of these pigments. The role of hydrogen sulfide in the production of these discolorations is one that is somewhat involved. Hydrogen sulfide reacts with reduced hemoglobin to form a purple compound. On exposure to oxygen, this purple compound oxidizes rapidly to form a green compound. Spectrophotometric measurements have been made on the green pigments obtained by the action of certain oxidizing bacterial enzymes on nitric-oxide hemoglobin and by the action of hydrogen sulfide on hemoglobin. These compounds were found to be spectroscopically different from methemoglobin. It will be remembered that methemoglobin is brown in color, not green. Hence, in speaking of the greening action of some bacteria on blood-agar plates, one should not designate the greenish halos as due to methemoglobin. Recent work indicates that these green pigments or "verdohemes" may be related to, or identical with, some of the bile pigments like biliverdin. The reader is referred to the publications of Lemberg and collaborators (61 to 67) for information on the chemistry and occurrence of some of these green pigments in various biological materials.

Green pigments, prepared by treating fresh ground beef myoglobin and fresh defibrinated hog's blood with H_2O_2 and $NaNO_2$ and also with H_2S, were fed to 25 white rats and 25 white mice, supplementing 20% of the green pigments in the standard basal diet. Each lot of test animals was fed these pigments for one month with no ill effects. These test animals were irradiated each week for 30 minutes under a General Electric S2 ultraviolet lamp; and they exhibited no untoward reactions to this treatment, i.e., there was no photo-sensitization induced by porphyrins as described by Blum (6).

What are commonly spoken of as "green rings" in sausage occur near the casing, varying in frankfurters, for example, in distance from the casing from one-sixteenth to one-eighth of an inch (1.5 to 3 mm), and in width from a faint—at times, discontinuous—circle to a band one-eighth of an inch (3 mm) across. The green discoloration may appear also as a core in the center of the sausage. The circular form of green ring has often been noted in square-pressed bologna. The various bacteria which can bring about these oxidizing reactions are: (a) those which elaborate oxidizing enzymes, some insensitive to catalase and thermostable, and some sensitive to catalase and thermolabile (effects destroyed by heating to 155–160F (68.4–71.1C) for 15 minutes); (b) H_2S-forming bacteria, both aerobes

and facultative anaerobes growing at an optimum zone in the sausage; (e) "oxidizing" bacteria growing at an optimum zone only. The ring formation is probably due to a phenomenon similar to that observed by Neill and Hastings (76) in which the oxygen tension governs the amount of oxidized hemoglobin in the presence of a constant amount of pneumococcus extract. Their data show that when the oxygen tension approaches zero, only traces of the oxidized pigment are formed by the extract; and when the tension of molecular oxygen is great, again only traces of the oxidized pigment are formed. However, at intermediate, optimum oxygen tensions much oxidized pigment is formed. In many instances, the green-ring formation in casing sausage is formed according to the quantity of oxygen dissolved, provided that the bacteria have grown sufficiently in the trimmings from which the sausage was made. The addition of sterile bacterial filtrates to fresh sausage materials has resulted in the production of green rings in the finished product. For a detailed discussion of these green rings, the reader is referred elsewhere (38).

The iridescence of sliced cured meats, such as ham, bacon, and dried beef, is due to the peculiar surfaces of cured meat fibers. The color is a structural color as opposed to a pigment color. It is due to the breaking up of white light by the highly fibrous character of the surface and to the film of fat on these fibers. If the fat is removed, the iridescence disappears. Any oil applied to the defatted surface will restore iridescence. The phenomenon has no sanitary significance, and is related to that of the diffraction grating or "clam-shell" play of colors.

2. ANTE-MORTEM AND POST-MORTEM CHANGES OF TISSUES OF FOOD ANIMALS

(a) *Bacteriology of Tissues of Living and Slaughtered Hogs.* There is very little information available as to the occurrence of bacteria in tissues and blood in healthy persons, according to Topley and Wilson (100). However, some writers (Norris and Pappenheimer (77), Arnold (3), and Burn (11)) state that there is definite evidence that tissues may not always be sterile in normal human beings. Most of the early work on the bacteriology of tissues of normal animals indicates that tissues, especially muscle, are sterile. Other workers have reported the presence of a variety of microörganisms in the blood, viscera, and muscular tissues of healthy animals (48). In 1926 Reith (82), after a careful study of various tissues of hogs and laboratory animals, came to the conclusion that aerobes and anaerobes are present in the musculature and blood of apparently normal animals. If one upholds the ante-mortem infection theory, the problem of meat spoilage becomes a refrigeration-engineering problem to a large extent, and whatever spoilage occurs must be considered unavoidable even though applied bacteriology functions ideally. However, the work of Burn (11) on post-mortem bacteriology points to the phenomenon of agonal invasion; and the work of Jensen and Hess (48) supports the view that the invasion of bacteria is agonal and post-mortem rather than ante-mortem. A series of biopsy studies was conducted on prime normal hogs whose blood, bones, bone marrows, and muscle tissues were examined bacteriologically. After the surgical fields were closed, sutured, and heavily covered with celloidin, the animals were immediately taken to the killing floor,

hoisted, stuck and bled, washed, dehaired, butchered, and dressed.   Before they were chilled and tanked, the control tissues and expressed blood were examined (40 minutes later).   Except for one animal (which appeared normal, but harbored *Hemophilus sp.* in all tissues examined while alive and after dressing as well), none of the hogs, while alive, showed the presence of microörganisms in the tissues or the blood.   The post-mortem findings indicated, however, that bacteria may occasionally be found in the tissues and blood.   The tissues post-mortem may show the presence of species of *Achromobacter*, *Pseudomonas*, *Serratia*, *Bacillus*, *Proteus*, *Micrococcus*, *Clostridium*, "diphtheroids," and *Torula* (48).   These findings suggest that bacteria may on occasion be found in tissues immediately after death from slaughter.

Many investigators have determined a few of the mechanisms which remove bacteria from the blood stream, a subject which has been reviewed elsewhere (48). Norris and Pappenheimer (77), who introduced *Serratia marcescens* into the oral cavity of human beings immediately after death, recovered these bacteria from the lungs several hours later.   Some pathologists say that contamination of the heart's blood occurs through the large veins from the lungs, and that heart's blood at autopsy practically always shows contamination with many species of microörganisms.   It has long been known by pathologists that, at autopsy, bacteria and remnants of food may be found in the lungs, i.e., "food down the wrong way."   It is not uncommon to find corn and other food in the lungs of slaughtered hogs.   Hülphers (33) describes microörganisms found in lungs of slaughtered hogs, and most of these microörganisms appear to be soil flora. There is a unique mechanism of contamination of some living animals shown first by Tarozzi (96) and later by Canfora (12).   Each of them injected spores of *Clostridium tetani* into animals.   There were no ill effects from these inoculations. After a considerable length of time (up to 55 days), a bone of one of the animals was broken or traumatized, following which tetanus usually occurred, showing the longevity of spores in the normal animal body.   Koser and McClelland (56) found the spores of clostridia recoverable from the body long after the spores of aerobes had disappeared.   Contamination of the bone marrows, especially the red marrows, may perhaps occur in the normal animal through permeability of the intestinal mucosa, via wounds, the lungs, and the upper respiratory tract. In this connection, the occurrence of latent infections should also be considered, Meyer (73).

Many theories, current in the meat-processing industry, on the source of contamination of tissues have been investigated (48), and brief mention is made of some experiments which may be deserving of further study.   The skin of a hog obviously is heavily contaminated with microörganisms.   When the stick knife passes through the skin, severing the jugular vein and sometimes the carotid artery, the blade is washed with venous and often with arterial blood.   The heart may beat from two to nine minutes after the stick wound is made.   Some of the shackled, hoisted hogs contract their heads in the direction of their forelegs and thus withhold some of the blood by constriction and hematoma, allowing some blood from this area to reach the heart and be circulated.   A "negative" pressure

may be set up in the severed or pierced vessels, owing to the labored breathing accompanying exsanguination (oxygen starvation). The flow of the pooled blood and blood within the vein is towards the heart.

Tests were made by dipping the blades in cultures of various bacteria and then sticking the hogs in the usual manner. Some of these bacteria (possessing an identifying cultural characteristic to distinguish them from the usual flora) were recovered from the marrow of the tibia and other bones. A large number of hogs were then bled aseptically (a technic which obviously brings to mind the methods employed by the serum companies which produce hog-cholera serum and other immunizing serums. It has long been observed that such "serum" hogs when slaughtered produce a very low incidence of sour meats). However, the aseptic bleeding as effected in the above tests did not close all portals of contamination of the tissues, including bone marrow; but technical sanitation of the pork-block in conjunction with other methods of applied bacteriology, reviewed elsewhere (48), effected a marked reduction in the numbers of sour marrows and tissues.

Practically all investigators in this field have observed the absence of coliform bacteria in hams. Boyer (8) states: "The absence of the *Bacillus coli* group of organisms from the numerous cultures taken from these hams is of particular interest. The members of this group are abundant and ubiquitous on the killing floor, and are almost invariably found on the surfaces of the carcasses which are exposed during killing floor operations. Their absence is of special significance in that it goes far to eliminate the possibility that organisms present in the hams gain access during killing floor operations."

The stick-blade contamination and the train of conditions following the severing of the neck vessels should favor the entry of coliform and other bacteria into the blood and marrow. However, some factor prevents their reaching the marrow or surviving therein if they do reach it. It is well known that undiluted, fresh mammalian blood is bactericidal (1). Hog's blood was drawn aseptically from the tail of a live hog, as is done by the commercial producers of serum, and bled directly into sterile containers containing glass beads for defibrination. Various freshly isolated strains of bacteria (*Escherichia coli*, species of *Pseudomonas, Serratia, Achromobacter*, vegetative clostridia and *Bacillus*, and *Staphylococcus aureus*) were added in minimum amounts of menstruum so that the blood would contain about 50,000 viable cells per milliliter. The defibrinated blood containing these bacteria was cultured at short intervals up to 24 hours. It was found that many of the flasks were practically sterile after 2 to 5 hours and other strains survived with less complete reduction in numbers. The ham-souring types of bacteria, such as certain strains of *Serratia, Achromobacter, Clostridium putrefaciens* of McBryde, and *Pseudomonas*, were more or less resistant to the bactericidal effects of hog's blood. The suspensions of *Staphylococcus aureus* and *Escherichia coli* were often sterile after 2 to 5 hours. These studies should be repeated and extended.

(b) *Autolysis of Tissues.* Over 70 years ago Hoppe-Seyler (32) recognized liquefaction of dead tissues occurring without accompanying putrefaction, and he noted that the phenomenon resembled the effects of digestive ferments. Some

experiments performed by Ernst Salkowski (88) in 1890 led this investigator to the discovery of tissue autolysis, which he named "autodigestion." He used chloroform as a bacteriostat. Little notice was taken of this work for a decade until Martin Jacoby (35) took up the work again and introduced the term "autolysis."

The one great difficulty in evaluating true autolysis is the uncertainty of continuing and effective antisepsis. Von Fürth (107) states that it was assumed there would be no danger of bacterial growth in finely divided tissues saturated with chloroform or toluol. He states that it was usually supposed that, if a few drops of toluol or a few bits of thymol were added to a thick emulsion of some tissue, it would be quite safe to incubate the emulsion for months, and that the danger of bacterial action "would be surely and for all time eliminated by such a purely symbolic performance (for that is actually about all it represents). . . . It is quite obvious why 'discoveries' prospered and multiplied in our literature on the same scale as did the bacteria in the pots and jars of the experimenters."

The question of tissue sterility also enters into the picture. There are times when many tissues of slaughtered animals are not sterile, although biopsy materials are usually sterile.

A large number of experiments have been performed to demonstrate the native autolytic enzymes of tissues (109). Some microbiologists do not subscribe to the existence of autolytic enzymes because of the faulty bacteriological techniques employed in certain biochemical experimentation. Recent work by Reeves and Martin (81) demonstrates that bacteria do grow in many tissue preparations containing preservatives calculated to stop bacterial growth. In their experiments, the sterility of digests could be determined only by smears and cultures. They repeatedly found bacteria growing in digests which were free from putrid odors and showed no evidence of bacterial growth. However, there are methods (2) available for the isolation of autolytic enzymes. Further work is needed in this field with new preservatives and more adequate microbiological controls.

There are several opinions extant on the exact mechanisms responsible for the tendering of meat. Some observers believe that the connective tissues are responsible for tough meat and that the change from collagen (of connective tissues) to gelatin correlates closely with the degree of tenderization. Other workers have concluded that connective tissues are very little changed and that the tenderness of meat is due to the muscle fibers becoming inelastic and thus tender. Another group does not consider that autolysis by native ferments plays any appreciable role in meat tendering during the first month of storage and does not reckon that microörganisms aid in the process.

The question of autolysis has been discussed in detail by Hoagland, McBryde, and Powick (30) who believe that meat tendering during storage may be regarded as largely due to enzyme action. There is no question that some denaturation of soluble protein occurs during rigor mortis. Denaturation, as well as autolysis of muscle, favors subsequent bacterial decomposition of muscle. Gibbons and Reed (21) studied the effects of autolysis on the subsequent bacterial attack in the muscle and kidney tissues of haddock. They found that the degree of autolysis,

preceding the introduction of bacteria, did not affect the growth rate but did make a marked difference in the subsequent chemical changes.

Ripening and tendering beef by aging in coolers with controlled humidities and temperatures is the common method employed in peace times in large meat plants. During the holding period, molds of several genera appear on the cut surfaces of the meat (usually, species of *Thamnidium*, *Rhizopus*, and *Mucor*). The bacteria found on the surfaces are usually species of *Achromobacter* and *Pseudomonas*. Hundreds of examinations of "whiskery" beef stored for tendering have revealed this flora. There is a sharp difference of opinion on the question whether or not microörganisms aid in producing the organoleptic qualities demanded of aged beef or whether autolysis produces these effects.

Under ordinary conditions beef is held about 5 days at 36° to 38°F (2.2° to 3.3°C) after slaughter before it is removed to various departments for final disposition. If the beef is to be held for "ripening" so that increased tenderness and flavor develops, it is observed that at higher temperatures shorter holding periods are required. For instance, beef held at the following temperatures and times show practically the same degree of tenderness (as judged by experts):

$$21 \text{ days at } 34°F \ (1.1°C)$$
$$8 \text{ days at } 40°F \ (4.4°C)$$
$$5 \text{ days at } 47°F \ (8.3°C)$$
$$3 \text{ days at } 60°F \ (15.6°C)$$

At the expiration of these holding periods at the various temperatures the meat should be handled promptly. There is no noteworthy difference in flavor of the lean meat, of the fat, or in the juiciness of the comparable steaks.

The prevention of shrink, preservation of bloom, and control of microörganisms on meats are subjects on which experiments go on continuously. The student is referred to the work of A. W. Ewell (15–18) for the practical effects of ultraviolet light and ozone on beef and foods in refrigerators of high humidity.

Many investigations have been made on the tendering effect of enzymes on sausage casings, ground meats, and other protein foods. Many enzymes have been used experimentally for this purpose. The enzyme of the osage orange (macin) is a strong protease, as is the asclepian from the juice of the milkweed. The action of the protease of edible mushrooms has long been observed when frying meats with fresh edible mushrooms. Ficin, the rapid acting protease of figs, can be used; and bromelin, the strong proteolytic enzyme in fresh pineapple juice, has been used for some time in tendering casings of frankfurters (80). Papain, the strong proteolytic enzyme of papaya, has been used in the Americas for a long time, Kilmer (52); and the proteolytic enzyme of the *Aspergillus oryzae-flavus* group can be used for any tenderizing of foods. None of these preparations can be used in inspected plants without permission of the Federal Meat Inspection Division.

The temperature at which such an enzyme becomes inactivated is very important; the ideal preparations are those inactivated at about 170F (77C). If the

enzyme is not inactivated at 165 to 175F (74 to 79.5C) during culinary heating the meat tends to become mushy or butyrous in texture which is organoleptically undesirable.

Enzymes are not used in tenderizing hams or in the preparation of ready-to-eat hams; to accomplish this end, the ready-to-eat hams are cooked in the smoke-house, and tenderized hams are heated in the smokehouse to an inside tempera-ture of 137F (58.4C) or higher.

3. ACTION OF MICROÖRGANISMS ON FATS

The action of microörganisms on oils and fats (other than butter fat) is a field awaiting extended study. Bacterial and mold metabolism of proteins and their derivatives and of carbohydrates have been studied extensively with brilliant results; but in the case of fat metabolism greater difficulties are encountered both in the few varieties of microörganisms that will grow on such substrates and the character of the split products. Again, many of the mechanisms of oil and fat "spoilage" have not generally been considered by oil chemists to be due to microbial action. To be sure, pure refined fats and oils freed from tissues and moisture are more prone to attack from atmospheric oxygen, light, and active catalysts like copper or iron than to direct contact with subsequent growth of microörganisms; but it has been proved in large-scale tests that fats, produced from animal and vegetable tissues that have been handled under the best sani-tary conditions, show greater stability than fats processed from tissues showing large numbers of lipolytic and oxidizing microörganisms.

Colin H. Lea, who has done extensive work on this subject, states (59): "It is only comparatively recently that oxidizing enzymes have come to be regarded as potential accelerators of oxidative rancidity in foodstuffs. The earliest work ap-pears to have been that of Jensen and Grettie (44), who inoculated fats with oxidase-producing organisms and found that the development of rancidity was frequently accelerated. The method they used for identifying oxidase-formers was to plate out the mixed organisms onto fat-emulsion agar and, after incuba-tion, to flood the surface of the medium either with dimethyl-p-phenylenediamine, or with the Nadi reagent. Colonies of oxidase-producing organisms treated in this way stained red or violet-blue, owing to oxidation of the reagent to a highly colored quinonoid compound. More recently similar colour reactions have been obtained from the soya bean and from pork adipose tissue, indicating that these too contain enzymic oxidizing systems. Such colour tests, however, only afford evidence of general oxidizing conditions, since hydrogen peroxide produced as a by-product of any enzymic oxidation would, in the presence of peroxidase, oxi-dize p-phenylenediamine and the Nadi reagent. It is, of course, quite possible that fat also might be oxidized by nascent hydrogen peroxide under these con-ditions.

"There seems little doubt, however, that certain types of micro-organisms do accelerate the production of oxidative rancidity in fats. Cases have been ob-served, for example, where beef fat stored under conditions favourable to the

growth of micro-organisms has rapidly lost its induction period (determined after extraction from the tissue), though separate experiment had indicated that the activity of the tissue oxidase was probably too low to account for this result."

Thoroughly dry, pure fats are incapable of supporting bacterial growth. However, when fats contain over 0.3% moisture it has been observed that microorganisms can grow on them. The constituents of fats other than glycerides may be important in rancidity. Jensen and Grettie (45) state: "Oils and fats from natural sources contain besides fatty glycerides small amounts of other materials, such as high molecular weight alcohols, hydrocarbons, protein residues and other nitrogenous matter, phosphatides and carotenoid pigments, which cannot be completely removed by any degree of refinement. Many of these minor constituents enter into and affect the reactions contributing to spoilage regardless of the source of the oil or fat. Their presence tends to confuse the study of the spoilage of oils and fats because they not only may decompose to small amounts of odoriferous substances but may act as catalysts, bacterial substrates, or inhibitors either retarding or promoting reactions contributing to the spoilage of oils and fats."

These writers conclude that microörganisms may cause (1) oxidative rancidity, (2) hydrolysis, (3) tallowiness, and (4) flavor changes owing to the production of various volatile products. It appears, therefore, that microbially induced rancidity is more extensive than has been realized.

The literature in this field, and the present status of the problems may be found in C. H. Lea's "Rancidity in Edible Fats," 1938 (58), H. K. Dean's "Utilization of Fats," 1938 (14), and L. B. Jensen's "Microbiology of Meats," 1942 (40).

4. EFFECT OF NITRATES AND NITRITES ON BACTERIA IN CURED MEATS

(a) *Action of Nitrates.* In the field of the microbiology of meats, there are few subjects over which there has been more controversy than on the effect of sodium nitrate on anaerobic bacteria in meats. Nitrate has for many decades been used in limited amounts together with sodium chloride to "cure" meats, both in pieces and comminuted. Nitrate is reduced by microörganisms in part to nitrite which forms by chemical action the heat-stable, nitric-oxide derivatives of the meat pigment, myohemoglobin. Since 1925, the Bureau of Animal Industry (now Meat Inspection Division) has permitted the use of sodium nitrite *per se* (not over 200 ppm) in meat-curing mixtures. It has been found, however, that nitrate is needed in the curing mixture and that there is much merit in the use of both nitrates and nitrites.

In 1907 Richardson (83) pointed out that "the most beneficial effect of nitrate in the curing of meat is its transformation of what would, otherwise, be *anaerobic conditions,* into aerobic ones in the bacteriologic sense. . . . I need only say here that it has been shown that aerobic bacteria will develop in the absence of air if nitrate is present in the culture medium and the anaerobes refuse to grow in the absence of air and presence of nitrate. The fact that typical putrefaction (Fäulnis) is an anaerobic process and that this process can be transformed into an

aerobic one by the presence of saltpeter, is a most important point in the curing of meat."

Grindley, MacNeal, and Kerr (23), in an extensive research on the effect of nitrate on bacteria in curing meats, found that in acid solutions (meats are usually at pH 5.9 to 6.1) even small amounts of nitrate exerted a marked inhibitory effect on bacteria in the curing vat. The growth of salt-tolerant bacteria was restricted by small amounts of nitrate in the pickling brine.

Summarizing his own work, Tanner (94) wrote: "While meat packers desired a nitrate cure a few years ago, results of extensive investigations indicate that, at times, a mixed cure (containing both sodium nitrate and sodium nitrite) is desirable from the standpoint of both spoilage and development of toxin by *Clostridium botulinum*. The preserving effects of such curing solutions have been generally attributed to the nitrites, either those added as such or those secured by reduction of the nitrate; however, evidence has been recently collected which might indicate that the nitrate itself may have some value as a preservative. This position is suggested by the fact that curing solutions containing nitrate have shown preservative action even though the nitrate reducers have been destroyed by the process."

Large-scale tests were performed in the Swift & Company Research Laboratories (36) on the effect of sodium nitrate[1] on spiced ham containing approximately 10,000 spores of *Clostridium sporogenes* per gram in 6-pound cans. The cans, and lots without nitrate added, were then processed for 5 hours at 68.3C (155F) according to a schedule no longer in use. The experiments showed that nitrate is of benefit in preventing the growth of *Clostridium sporogenes*. It was also observed that spores of some species of *Clostridium* are killed at lower temperatures and at shorter holding periods when heated in the presence of 0.1% $NaNO_3$. This adjuvant effect of nitrate was investigated by Yesair and Cameron (114) who found that the effect of curing salts on the thermal death time of spores of the test organism is apparently to reduce the time at temperatures lower than 230 to 235F (110 to 113C), and the slopes of the thermal death-time curves are greater when the salts are added. This phase of the subject is deserving of further study. When spores of *Clostridium botulinum* are incorporated in cured meat and thermal death-times compared with those of the spores incorporated in uncured meat, the inhibitive effects of the curing salts are apparent, but when the heated meat is subcultured in a liquid medium the spores are shown to be viable. Resistance values determined by subculture after heating are approximately the same in the cured and uncured meats. One of the shortcomings of nitrite is that it reacts with protein when heated at canning temperatures and is destroyed, and thus if nitrate is not present the meat in cans might not be well protected from germination of resistant spores of clostridia.

The sensitivity of species of *Clostridium* to oxygen still awaits satisfactory explanation (91). It is held by some bacteriologists that in the absence of catalase much H_2O_2 can be formed by many bacteria or accumulates, and that the amount

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[1] To each 200 lbs. meat were added 4 oz. (0.125 %) $NaNO_3$, 0.5 oz. (0.015 %) $NaNO_2$, 3.5% NaCl, 2% sugar, spices.

of peroxide formed might kill vegetative forms of sporing anaerobes or retard their development. This view has been objected to on the grounds that prolonged aeration of culture media containing material oxidizable by these bacteria does not form H_2O_2. Also when catalase is added, certain clostridia do not grow aerobically. Again, it is held that washed suspensions of *Clostridium sporogenes* aerated in an oxidizable medium fail to take up oxygen.

Leifson (60) found, however, in extended experiments on spore-forming bacteria, that only vegetative anaerobic cells were oxygen sensitive. These cells were so sensitive that most of them were killed by less than 2 to 3 minutes of exposure to air. The change from the nonsensitive spore to the exceedingly sensitive vegetative cell was so abrupt that only in a few cases was the sensitivity of the intermediate stages obtained. His results also show the well-known effect of nitrate on the germination of bacterial spores. He found 1% sodium nitrate to inhibit sporulation of *Clostridium botulinum*.

McLeod and Gordon (72) obtained their evidence of peroxide formation by observing greening of heated blood agar (chocolate agar) a few millimeters below the surface at a zone where penetrating atmospheric oxygen meets with growth of clostridia. It is possible that the greening action is due to production of thiol compounds which may, on contact with oxygen, form H_2O_2. On the other hand, H_2S may form sulfhemoglobin or related compounds which, upon contact with oxygen, change into the green compounds described in Section 1.

When *Clostridium botulinum* is grown under anaerobic conditions on the surface of blood-agar plates containing benzidine and then exposed to air, sufficient peroxide accumulates to produce dark halos. This test shows peroxide production in the presence of oxygen. Hence, since strict anaerobes apparently do not produce catalase, the organisms are unable to destroy the toxic oxidizing compound. (Peroxidase does not decompose H_2O_2 in the absence of an oxidizable substance, whereas catalase decomposes H_2O_2 in the absence of an oxygen acceptor.)

The work of Hart and Anderson (26) indicates that the green discoloration produced on chocolate agar can be reproduced anaerobically by the action of reducing systems, including cysteine and bacterial suspensions with hydrogen donators. The identity of the green pigment is not clear, but the above evidence is taken by many writers to prove that hydrogen peroxide is not the only "reagent" which can form a green zone in blood pigments. Hart and Anderson state that discoloration of heated blood agar (chocolate agar) by certain streptococci is inhibited by catalase which is direct evidence that the greening is due to H_2O_2. The greening action on unheated blood media by the pneumococci and streptococci is viewed by them as an entirely different phenomenon. The discussion in Section 1 may extend some of these views.

The reader is referred to the work of Broh-Kahn and Mirsky (9) who do not agree with McLeod and Gordon's peroxide theory. They believe also that the reduction potential theory of Quastel and others is equally untenable. It may be concluded that no theory at the present time explains why anaerobes cannot grow in air, and in the writer's opinion, meat technologists should reserve judg-

ment as to the effect of nitrates on clostridia and the mechanisms of green-pigment formation until this field is more fully developed.

The important work of Tarr (98) on the action of nitrite on bacteria, recently reported, needs serious attention in evaluating the mechanisms of growth retardation of anaerobes and aerobes in mixed cure with nitrate.

(b) *Action of Nitrites.* Tarr (98) has shown that nitrites may, under certain conditions, play an important part in retarding the growth of many kinds of bacteria and thus delay the spoilage of meats. The work done by other investigators has indicated that large amounts of nitrite—greatly in excess of 0.02% nitrite permitted by law—are necessary to kill or inhibit bacteria. Since previous workers did not state the pH of the media they employed, it may well be that the media were neutral or faintly alkaline. Tarr found that bacteria are susceptible to low concentrations of nitrite only at pH values below 7. Not all bacteria studied proved equally susceptible to nitrite, and certain organisms proved to be resistant. The growth of species of the following genera at pH 5.7 to 6.0 was either inhibited or prevented by 0.02% of sodium nitrite: *Achromobacter, Flavobacterium, Pseudomonas, Micrococcus, Escherichia, Aerobacter,* and one species of *Torula.* Tarr found that in the acid range 0.02% $NaNO_2$ also inhibited the growth of two species of obligate anaerobes, *Clostridium botulinum* and *Clostridium sporogenes.* Likewise, 0.02% $NaNO_2$ inhibited the growth of *Eberthella typhosa* and *Staphylococcus aureus.* While Tarr does not attempt to explain the mechanisms of inhibition, he points out that nitrites may combine with respiratory hematin compounds. Ingram (34) found that oxygen uptake in the case of the aerobic, cytochrome-containing *Bacillus cereus* is inhibited by traces of nitrite. There is little doubt that nitrite acts directly upon the bacterial cell, but whether its action is general or specific is not clear, according to Tarr. He found that $NaNO_2$ in concentrations of 1 and 10% exerted marked bactericidal action in acid medium, but not above pH 7. In this connection, Bittenbender *et al.* (5) reported that *Staphylococcus aureus* was not killed upon exposure to 38.8% of sodium nitrite for 10 minutes at pH values between 3 and 8. These workers did not report the numbers of organisms inhibited but recorded only the presence or absence of growth.

Quastel and Wooldridge's (79) work suggests that nitrites inactivate certain enzymes by combining with their amino groups.

The theory that inhibition of the growth of sporing anaerobes during meat-curing processes is due to H_2O_2, a substance which is toxic for clostridia even in extremely small amounts, and which may accumulate through inhibition of catalase by the hydroxylamine formed in cures (46), may well account for some growth inhibition; but Tarr's phenomenon shows that nitrites also retard clostridia under such conditions. Some recent work has demonstrated that hydrogen peroxide does possess antibacterial action. Green and Pauli (22) have observed that the antibacterial action of the xanthine oxidase system is due to the formation of H_2O_2, a product of the enzymic action. Van Bruggen, Raistrick, *et al.* (105) have shown that penicillin B (an enzyme of flavoprotein nature) owes its bactericidal powers to H_2O_2, a product of the enzymic action. McCulloch

(70) writes that H_2O_2 can prevent the growth of anaerobic organisms although its influence is transitory, but it is incapable of destroying the spores of anaerobes.

When nitrite in meat is heated at canning temperatures above 100C (212F), much of the compound is destroyed. Brooks, Haines, Moran, and Pace (10) have observed that at 212F the time required for fifty per cent destruction of nitrite increases with the initial concentration ranging from 13 to 120 minutes for values of 30 to 589 g $NaNO_2$ per 10^5 g tissue. With the usual times of cooking, the reduction from a high to a low nitrite content cannot be expected.

The presence of nitrate in a finished sausage or other mixed-cure product on occasion presents the disadvantage of continued production of nitrite by bacteria during transit to some inspection laboratory or during improper storage in the chemists' locker before undergoing analysis. Much misunderstanding has arisen from this avoidable bacterial action, and cured meat foods showing over 200 ppm of nitrite upon ultimate analysis have undoubtedly often left the sausage kitchen with the lawful limit of nitrite present. It is not clear how the nitrate-reducing bacteria can grow in the presence of such large amounts of nitrite found in experimentally incubated, mixed-cure sausage.

It is possible that the characteristic flavor of bacon and ham (as opposed to salt pork) is due to a product of a reaction of nitrite with constituents of the tissue, either during curing or during cooking, according to Brooks, Haines, Moran and Pace (10).

(c) *Action of Nitrates on Gas Formation by Species of Bacillus.* The typical gaseous swell of canned cured meats is practically always due to the fermentation of sugar by species of the genus *Bacillus*. Large quantities of carbon dioxide are formed when either the small or large varieties (except Donker's group) grow in a medium containing fermentable carbohydrates, nitrates, and cured pork meat, or in "sugar-cured meats" at temperatures ranging from 23.9 to 48.9C (75 to 120F). This gaseous fermentation also produces spongy beef hams and causes bursts in large bologna. None of the hundreds of strains examined can form gas in the ordinary sugar broths or sugar-agar media.

Nitrate, sugar, and cured meat must be present together before the bacilli ferment with gas production. If the medium contains c.p. nitrite without nitrate, no carbon dioxide forms. If sugar is omitted, no carbon dioxide, or very little, is formed. If cured meat is omitted and nitrate, sucrose, and any ordinary soluble proteins or peptones are added, no carbon dioxide forms. With some strains of the bacilli, gas is formed if nitric-oxide hemoglobin or nitric-oxide hemochromogen is present in the sucrose-nitrate medium. Some strains of the genus *Bacillus* do not form acid in the standard sugar-veal infusion broths (47) but do form acid and carbon dioxide when grown in nitrate sugar-cured pork medium. This was observed with the disaccharides and one of the pentoses, xylose. In the case of sucrose-nitrate-cured spiced ham carbon dioxide is formed as follows: The first reaction observed is the splitting of some of the disaccharides to monosaccharides. Later, lactic acid is found in appreciable quantities and also much carbon dioxide. In the closed container, as used for canned meats, where there is no escape for acetaldehyde, much 2,3-butylene glycol is formed. The order ap-

pears to be disaccharide to hexoses to triose compounds and then to pyruvic acid. A part of the pyruvic acid is reduced to lactic acid and a part is split into acetaldehyde and carbon dioxide. The acetaldehyde may either be reduced to alcohol, oxidized to acetic acid, or condensed to form acetyl methyl carbinol which can be reduced to 2,3-butylene glycol.

We have never observed the formation of carbon dioxide in media other than nitrate-sugar-cured pork meat or nitric-oxide myohemoglobin or hemochromogen except in a few instances where thiamin was added to the nitrate-sugar medium. Presumably, phosphorylated vitamin B_1 acts as a coenzyme which decarboxylates pyruvic acid; and there is a comparatively large amount of thiamin in pork. Thiamin is not a complete factor in this mechanism since gas was not observed to be formed by many strains of bacilli capable of gaseous fermentation in the nitrate-sucrose-cured pork medium (47).

The spores of this group are killed by the present-day processing schedule for canned spiced ham or luncheon meats. In the presence of 3 to 3.5% NaCl, 2.33 oz (0.15%) $NaNO_3$, and 0.125 oz (0.008%) $NaNO_2$ per 100 pounds of meat, the 6-pound cans may be inoculated with spores of clostridia, bacilli, or thermophiles and retorted at 112.8C (235F) for 3.5 hours so that an inside temperature of 107.2C (225F) is reached with a finished product that will keep at temperatures below 55.5C (132F).

ZoBell (116) has demonstrated the ability of some microörganisms to destroy nitrites as they are produced from nitrates, and recommends that tests for nitrates be made in conjunction with tests for nitrites when the latter substance is not found. In this connection, we have observed that hydroxylamine may be found in the old-style, long-mixed cure for hams towards the end of the curing period (60 to 80 days). The present-day ham is cured in less than 20 days. Lindsey and Rhines (68) have demonstrated the production of hydroxylamine from the reduction of nitrates and nitrites by various bacteria. Hydroxylamine hydrochloride, when added to nutrient agar, inhibits the growth of food-poisoning varieties of staphylococci in dilutions of 1:20,000, and many of the common molds in dilutions of 1:15,000. Kitasato (53) has reported that hydroxylamine when added to culture media inhibited the growth of *Clostridium tetani*, *Clostridium chauvei*, and *Clostridium septicum*.

5. ACTION OF SALT (NaCl) ON BACTERIA

(a) *Action of Salt on Bacteria in Meats.* For the past half century the demand for heavily salted foods has lessened in many parts of the world. Salt-preserved fish and meat were for a long time staple articles of food in world trade, and the preservation of foods by salting has proved its efficacy since time immemorial (55). The New England-West Indies trade in salted meat was of importance in colonial times (99).

Lessening the amounts of salt used in food preservation has resulted in changes in microbiological flora and brought forth many phenomena of interest to the bacteriologist. Tanner and Evans (95) have discussed the literature on the effect of salt on pathogenic bacteria and stated that most investigators indicate that

salt in the concentrations used in food preservation is not a bactericide but acts as a preservative by inhibiting many species of bacteria. Tanner and Evans conclude from their own extensive experiments that salt is the most active preservative used in meat-curing solutions.

Petterson (78) has shown that the preserving action of salt in meat and fish is not sharply defined. Salt exhibited a selective action and did not inhibit all bacteria. Anaerobes were inhibited by 5% NaCl whereas aerobic rods, facultative anaerobic rods, and micrococci were not greatly inhibited. The rod forms were more easily suppressed by salt than the cocci. Ten per cent NaCl appeared to inhibit most bacteria, although a few grew in media containing up to 15% NaCl. Various yeasts appeared to persist and grow in media containing over 15% NaCl.

At this point attention is called to the diverse results which can be observed when, for instance, 5% salt is used in pickling meats or is added to liquid culture media. Tanner and Evans (95) have demonstrated that the inhibitory effect of salt on *Clostridium botulinum* is different when the same concentrations of NaCl are used in the different culture media. Glucose agar containing 6.5% or more of NaCl did not support growth. Nutrient broth containing 6.7% NaCl allowed one culture to show visible signs of growth and five strains of *Clostridium botulinum* to grow and produce toxin. It was necessary to add 7.8% NaCl to prevent the growth of all strains in nutrient broth. When 7.3% NaCl was used in glucose broth and 7.8% in pork-infusion broth, growth and toxin formation took place. Growth took place in 10.5% NaCl in glucose broth and 12% was needed to inhibit growth in this medium. With cooked pork toxin formation was prevented by 5% NaCl.

When over 3.5% NaCl is used in curing whole pieces and comminuted meats, most anaerobes are suppressed although there are a few strains of nonpathogenic, sporogenous anaerobes that can germinate and multiply when given heat treatment several times greater than that necessary to destroy spores of *Clostridium botulinum*. Fortunately these strains are encountered very infrequently and at this time are a curiosity in culture collections.

Bacillus foedans of Klein, which we have equated with *Bacillus putidus* of Weinberg (48), is inhibited in hams containing 3% NaCl and undergoing the smokehouse processing. This organism is very proteolytic and grows in nonpickled areas, sometimes formed through by-passing the bifurcation of the iliac artery during pumping. This condition may be differentiated from sarcosporidial activity, which likewise causes a butyrous area, but is associated with a normal salt content and normal organoleptic characteristics.

Many species of aerobes and facultative anaerobes (as well as strict anaerobes) can grow in high salt concentrations in brines which contain large pieces of various animal tissues; but the growth takes place at the interfaces of brine and tissues and not readily in the clear brine. When meat infusions or whole blood are added to 15 to 20% clear NaCl brines, the growth of salt-tolerant microörganisms is very slow. When large pieces of meat are added to these vats and the salometer readings are kept constant, growth proceeds more rapidly, usually on the surfaces of

the meat. The mechanisms of growth at the interfaces appear not to be well understood at the present time.

In the curing of comminuted meats, the ratio of salt to total moisture obviously accounts for the low quantities of salt which suffice to inhibit many microorganisms. For instance, in composite beef muscle the water content is about 71% and protein 18.7% with a water-to-protein ratio of 3.8, while in lean pork the water content is 64.5% and protein 18.9% with a water-to-protein ratio of 3.4 (74). A higher ratio of salt to water accounts for the preservative effect of salt in meats as compared to liquid culture media; but the problem of free and bound water in tissues confronts one here as in so many other fields of food technology. Long experience has taught the curing departments of the industry the precise amounts of salt necessary (in addition to the lawful amounts of nitrates and nitrites) to be effective in each of the many kinds of meat foods.

(b) *Effect of Impurities in Salt.* A phase of the salt curing of meat which has received attention from time to time is the effect of impurities in the salt. Many commercial meat processors are of the opinion that impurities in common salt (calcium, magnesium) affect the meat adversely, whereas some believe that more desirable cures, from the standpoint of rapid penetration, are affected. Moulton and Lewis (75) made up sodium chloride curing solutions containing 5% $CaCl_2$, or 1% $CaCl_2$ + 1% $MgSO_4$, or 1.5% $CaCl_2$ + 0.5% $MgSO_4$ for curing hams, butts, and beef knuckles. They found no consistent effects of these impurities in sodium chloride brines upon the rate of penetration of the NaCl into the meat. They concluded that commercial salts would show no measurable differences in penetration rates owing to the usual impurities.

There is an extensive literature on the physiologically antagonistic effects of ions (20). There is evidence that the toxic action of an ion can be nullified by another different ion. In the case of bacteria there is a quantitative antagonism but the qualitative effects of all cations may be alike. The toxic effect of a monovalent salt like NaCl can be neutralized by the addition of a divalent salt in suitable proportion. Winslow and Falk (112) have found that 0.145 M $CaCl_2$ + 0.290 M NaCl was toxic to *Escherichia coli*, but a solution of 0.145 M $CaCl_2$ + 0.680 M NaCl was nontoxic.

Tressler and Murray (102) have noted that the presence of calcium and magnesium compounds as impurities in salt accelerated the development of the salt-fishy odor and taste of salted fish. It has been observed that if pure salt was used for the salting of fish, the product when thoroughly freshened was nearly indistinguishable from fresh fish. Tressler (101) had observed that impurities in the salt exerted a depressing action on the permeability of the cell walls and thus slowed down the penetration of salt into the muscle tissues. The various effects of salt impurities have been studied by E. Hess (28) who states: "Spoilage, as measured by rise in trimethylamine and bacterial content, of cod press-juice containing 21 grams salt per 100 ml. (about 80% saturated) is delayed longest with pure NaCl, and increasingly less with mined evaporated, mixed crude, Mediterranean and Turk's Island solar salts. This order corresponds to decreasing percentages of NaCl and increasing percentages of impurities in these salts. The

differences in the salt action increase with lower temperatures." Hess used fish-muscle press-juice instead of the muscle itself which eliminates the penetration factor and also makes bacterial counts more accurate. Hess quotes unpublished data of Gibbons who observed that a medium not containing calcium or mag-nesium ions (prepared from dialyzed drip of fish muscle, washed agar, and pure NaCl) did not support the growth of red halophilic bacteria, or only very poorly, whereas the addition of traces of calcium or magnesium or replacement of the sodium chloride by solar salt resulted in good growth. The same effects can be observed on cells of red halophilic bacteria in the presence of $CaCl_2$, $MgCl_2$, and $MgSO_4$ in high concentrations of NaCl. Beef cured in 100° salometer brine with solar sea-salts and stored at 32.2 to 37.8C (90 to 100F) will spoil with character-istic trimethylamine odors after 5 months, showing a typical flora of *Serratia*, *Achromobacter*, and *Micrococcus*. The work of Tarr (97) is interesting in this connection in that he has discovered an enzyme, "triamineoxidease," existing in cells of five different genera of bacteria which can form trimethylamine.

The dry-curing process employed to cure country hams and southern-style hams is interesting from the standpoint of salt permeability. In observing this practice in many places in America, it was noted that the farmer or commercial processor first rubs sodium nitrate over the ham, usually rubbing from the shank towards the butt. The shank (tibia and fibula) is "sawed long" to effect marrow sealing. The nitrate appears to increase permeability or "cause swelling and a moist condition." Then after a time salt or salt and sugar are rubbed into the ham in the same manner as the nitrate. The process is repeated a number of times for about seven weeks, and then the hams are smoked for varying lengths of time. The effect of nitrate on the permeability has not been given much sci-entific study (110), but the art of curing in this manner is very old. Surprisingly *few pieces cured in this way show frank spoilage (as distinguished from incipient "souring").*

It is known that very dilute nitric acid softens the connective tissue binding the muscle fibers. This action may aid in "tendering" certain cured meats. Studies are needed on the action of nitrates, nitrites, salt, sugar, and enzymes on muscle fibers, sarcolemma, sarcoplasm, myofibrils, discs, and other structures of muscle.

Rockwell and Ebertz (86) conclude that the preserving effect of NaCl involves more than its dehydrating capacity. For instance, if common salt inhibits bacterial growth by means of dehydration, then other equally efficient dehydrat-ing salts should serve as inhibitors. Magnesium sulphate has greater dehydrat-ing effect on proteins than NaCl but is not as efficient in preventing the growth of *Staphylococcus aureus*. They conclude from their experiments that the preserv-ing action of NaCl on proteins involves more than dehydration, there being four other factors operative. These factors are: the direct effect of the chloride ion, removal of oxygen from the medium, sensitization of the test organism to CO_2, and interference with the rapid action of proteolytic enzymes.

(c) *Bacteria in Salt.* From time to time, salt contaminations leading to spoilage have been suspected in meat processing, but no unanimity of opinion exists either among food bacteriologists or operating men in the industry concern-

ing the hazards of contamination in salt. There are even those food bacteriologists who doubt the existence of obligate halophiles as spoilage organisms, and who view these bacteria as salt-tolerant forms. The distinction is not clearly made in some of the literature on salt. The observations of Clayton and Gibbs (13), Robertson (85), and ZoBell, Anderson, and Smith (117) indicate that halophilic bacteria are distinct species indigenous to environments containing high concentrations of sodium chloride. ZoBell *et al.* (117) found an average of 167 obligate halophiles in Great Salt Lake water having a salinity of 27.6%. These halophiles required at least 13% salt for growth. These bacteria are believed to have become acclimatized to increasing salt concentrations during the time that the water of old Lake Bonneville evaporated to leave its saline remnant, Great Salt Lake.

The studies of Stuart, Frey, and James (92) and Stuart and Swenson (93) indicate that halophilic bacteria are adapted forms of ordinary bacteria known to be indigenous to other environments, such as soil, etc. Lochhead's work (69) on halophilic bacteria should be consulted in this connection.

The data obtained during the past decade (37) from microbiological analyses of mined salt and vacuum-pan salt of various grades indicate strongly the improbability of contaminations in salt sufficiently important to lead to meat spoilage. Certain of the solar sea-salts may, however, need careful scrutiny. Yesair (113), who examined many grades of salt, advised microbiological examinations of the salt as a control measure in the food industry.

The question of the nature and origin of halophiles has been reviewed elsewhere (37).

(d) *Some Effects of Salt on Bacteria.* Large initial numbers of contaminating bacteria tend to grow in meats that are highly salted. The observation has been made many times that large numbers of non-halophilic or non-salt-tolerant bacteria tend to neutralize the inhibitory effect of salt. It appears that the larger the inoculation the greater the salt tolerance of the bacteria. The work of Sherman and Albus (89) shows that when active reproduction takes place there is marked destruction of the cells in the presence of 5% NaCl. This observation is borne out in practical application where it is noted that fresh meats showing light bacterial contamination keep better when salted quickly than when held for a time before salting. The old cells are not as sensitive to salt as the actively growing cells.

The effect of sodium chloride on thermal death times of bacteria has been studied by Esty and Meyer (19) who found that NaCl in low concentrations (0.5 to 1%) markedly increased the thermal resistance of spores of *Clostridium botulinum*. At 2% concentration of salt, this effect was lost. Up to 8%, little or no effect of the salt was observed. Above this concentration, up to 20% NaCl solution, the effect was to decrease the thermal death times. Viljoen (106) studied the adjuvant effect of NaCl upon thermal death times of spores in pea liquor. He found a protective influence of salt in the range of 1 to 2.5% NaCl, and 3% salt shows about the same protection as 0.5%. Four per cent NaCl increases very slightly the destructive effect of heat.

In the case of micrococci, salt appears to exert a protective action against the effects of heat. Alkaline solutions of salt reduce the thermal death times of spores in a very striking manner.

The terms "obligate halophile," "salt tolerant," "salt resistant," and "facultative halophile" may all be employed to indicate the characteristics of a strain, but there is still difference of opinion as to the distinction between such terms. If Kluyver and Bahrs' (54) doctrine of "physiological artifacts" is true, i.e., that halophilism is developed by conditions of artificial culture in saline media from a prepotency latent in the cell of the saltless environment, much remains to be done before the terminology becomes clear. They demonstrated the interconvertibility of nonhalophilic *Microspirum desulphuricans* (Beijerinck) and halophilic *Microspirum aestuarii* (Van Delden) by gradual alteration of the salt content of the medium. They suggested that these forms are all derived from a common parent strain, and hence the specific identity of the halophiles and non-halophiles. F. B. Smith has written an excellent review of this subject (90).

There are many strains of obligate halophilic molds, usually divergent forms of brown molds, that exhibit all degrees of salt tolerance. Some are psychrophiles, but most strains are mesophiles. Some resemble species of *Torula*, others resemble species of *Hemispora*, *Oospora*, or *Sporendonema*. *Aspergillus candidus* will grow on salted smoked meats and produce small reddish patches.

6. Some Recent Developments in Meat Technology

The war has brought about some interesting developments in meat technology as well as in food technology generally. The outstanding developments in the production of foods on a scale hitherto undreamed of have been along the lines of dehydration of pork, beef, eggs, milk, soups, and some poultry meats. Boneless, well-trimmed beef (produced under bacteriological control) and other meats are frozen; and through the efficient handling of the Quartermaster Corps and Veterinary Corps, these meats are made available for consumption in battle zones. The microbiology of freezing meats and of all kinds of stored fresh and cured meats and frozen eggs has taken on new life. Bacteriological studies have shown that there are critical storage temperatures at which the "indigenous" flora die off rapidly. For instance, we (42) have shown that frozen egg magma inoculated with several strains of *Escherichia coli*, several species of *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Micrococcus*, when frozen, divided into two lots, and then held at -22 F (-30 C) and 22 F, (-5.6 C) shows sharp differences in reduction of all bacteria in four weeks. Strangely enough, the bacteria are reduced to very low numbers of viable cells at 22 F; and there is very little or no diminution of viable cells to be found at -22 F. There are also critical holding temperatures for beef, pork, lamb, veal, and dressed poultry. Atmospheric oxidations complicate some of the benefits derived from the "higher" temperatures of storage.

(a) *Dehydration of Meat.* Processes for dehydration of meat of all kinds have come to the fore during the war years, and the reader is referred to the excellent work of von Loesecke (108) for details of the drying and dehydration of foods. Four kinds of meat have been prepared: fresh meat cooked and raw, and cured

meat cooked and raw. Most of the dehydrated beef and pork of commerce is pre-cooked fresh meat dried to 10% moisture and then packed and sealed in tin cans. Extensive studies carried out by the U. S. Department of Agriculture and the meat packers have shown that from a bacteriological standpoint dehydrated meat, made in accordance with Federal specifications, is safe and will remain so when stored without refrigeration in hermetically sealed containers (57). In such dehydrated meat, we notice a rapid decline in the numbers of bacteria. The pH is 5.8 to 6.2. *Salmonella sp.* staphylococci, *Clostridium botulinum*, and other pathogens will not grow in this product until the meat is rehydrated to contain over 30% moisture.

Commercial desiccation of foods is no new thing. During the Civil War troops were supplied with desiccated vegetables, soup mixtures, apples, peaches, etc. General Sherman wrote in his memoirs: "During the Atlanta campaign we were supplied by our Commissaries with all sorts of patent compounds, such as desiccated vegetables, and concentrated milk, meat biscuits and sausages, but somehow the men preferred the simpler and more familiar forms of food, and usually styled these 'desecrated' vegetables and 'consecrated milk'." Dehydrated rations were used in the Boer War; and the A. E. F. veterans of 1917, who accounted for the consumption of 10 million pounds of desiccated foods, exhibited about the same attitude as their grandfathers towards these dried foods. Following World War I the dehydration of foods was not extensive, but with the coming of World War II this business has taken on new life. Improved methods may save the industry from complete collapse after the war ends.

An interesting application of rapid dehydration of meat has been employed by Ritchell, Piret, and Halvorson (84) to form products like dry or summer sausage. They investigated air-drying of *uncooked cured* meats. In contrast to the usual sausage drying operations, shorter times of drying were necessary to obtain a product containing 25 to 30% moisture.

Work done by Dr. E. E. Rice and Dr. H. E. Robinson of the Research Laboratories of Swift & Company shows that:

Protein quality is not significantly reduced during dehydration or canning unless diets very low in proteins are considered.

Vitamin retention of pork and beef undergoing dehydration or canning is similar to that for household cooking of similar meats, being thiamin, 60 to 70%; riboflavin, 90 to 100%; niacin, 90 to 100%; and pantothenic acid, 70 to 80%.

Cured pork undergoing commercial canning (12-oz. can) retains 67 per cent of its thiamin, 90 per cent of its riboflavin, 94 per cent of its niacin, and 76 per cent of its pantothenic acid. Thiamin retention in 6-lb. cans is lower, being 40 to 50%.

During storage of either canned pork, dehydrated pork or dehydrated beef at temperatures up to 99 F (37.2 C) there is little or no loss of niacin, riboflavin, or pantothenic acid over a period of 219 days. Above 120 F (48.9 C) there are slow losses of riboflavin and pantothenic acid. Thiamin decreases more rapidly, showing some loss at 80 F (26.7 C). After 293 days' storage the thiamin retention in canned pork is 52 per cent. In dehydrated pork the retention is poorer, being 28 per cent after 219 days at 80 F (26.7 C). At higher temperatures there is almost complete destruction of thiamin in both products.

Molds can develop on dehydrated meats when these foods are exposed to the atmosphere and stored at relative humidities below 75% in the temperature range

of 10 to 37.2 C (50 to 99 F). Molding is, however, not a hazard in the tins and other containers used in distribution of this product.

(b) *Bacterial Standards.* The bacteriological control of foods by the criteria of agar-plate counts has long been a "standard method" for the inspector and producer. Most bacteriologists have been confronted with the dilemma of evaluating a food product from the "count" or the more laborious examination.

The subject of bacterial counts, i.e., bacteria growing into colonies in agar plates when incubated in the range 20 to 37 C (68 to 98.6 F) for varying lengths of time, as criteria for judgment of many foodstuffs is controversial at the present time. One school of thought would throw over the existing agar-plate count standards for most foods and replace this method by qualitative bacteriological examinations together with organoleptic criteria and chemical examinations. This group claims that when, let us say, a food like dried eggs contains 500,000 "viable aerobes" per gram and the "standard limits" are 250,000 per gram, the egg powder should not be rejected as inedible. There is undoubtedly much to be said for this position. However, the regulatory laboratories must have some criteria and limits of numbers if their investigators are to rely on "standards of numbers of bacteria permissible." The total number of bacteria growing on agar plates do, in many instances, serve to guide the processing of foods so that incipient spoilage does not result.

In meat foods, for the most part, it is more important to know both the kinds and numbers of the flora present than to know merely the numbers of microorganisms. Because most bacteria which grow in meat under refrigeration are psychrophiles or facultative psychrophiles, such as many species of *Achromobacter*, *Pseudomonas*, or *Serratia*, it may be considered that a "high count" does not necessarily indicate that the food is unsound if the organoleptic characteristics are satisfactory. Again, if counts are made on certain sausages, such as Thuringer, the number of lactobacilli found is very great indeed (40).

In the preparation of frankfurters, bologna, meat loaves, and other comminuted meat foods, the bacteriologist must be guided by qualitative bacteriological data. The total agar-plate count cannot presage the possibility of oxidizing effects leading to discolorations.

Meat, when produced under Federal inspection, is not likely to harbor pathogens, according to the data of the Bureau of Animal Industry, the Meat Inspection Division, or the Research Laboratories of the Meat Industry. There are no "normal" plate counts for meats in the literature available to us. A satisfactory count on meats may depend upon whether or not the meat is destined for use in sausage or, in the case of beef, whether or not it is to be aged in coolers for some time to induce tenderness, flavor, and juiciness. As there are several hundred kinds of meat foods produced continuously, no simple answer is forthcoming in respect to a "single standard" for these foods. It would be helpful if we could state with conviction founded upon experience that liver sausage should not exceed sixty thousand aerobes per gram or that pork links or patties should not exceed several hundred thousand aerobes per gram. However, perfectly edible sausage might show three times these figures, depending upon the length of time the food has been stored in the shop or commissary refrigerators.

It would appear that the ideal laboratory examinations of a finished food product should disclose the presence or absence of pathogens or toxins, and that both plate counts and determination of flora may be used in control of the processing. Methods for direct microscopical counts of foodstuffs are not wanting in number. Some inherent difficulties in direct-count methods are: (a) numbers less than 1×10^5 organisms per gram in food substances, such as muscle, cannot be accurately counted; (b) dead cells are counted (this is not a serious error); (c) errors in sampling, preparation, grinding, smearing, etc., are not eliminated; (d) "primitive" forms are not detected (41), nor are types determined except in a very general manner. Other shortcomings are perhaps apparent to the analyst, but in theory the method of direct count obviously has much to commend it. However, the problem is not considered impossible of solution, and the direct count could supplant the agar-plate count in many places.

(c) *Hams.* New applications of old methods of establishing bacteriological criteria for the curing and smoking of hams (not tenderized, not ready-to-eat) have been effected and incorporated into official specifications. These applications are based on the bacteriological observations showing that cured hams should be smoked at temperatures below 125 to 130 F (51.7 to 54.4 C) for several days for the smoke to penetrate well, that some dehydration should occur, and above all, that the tissues do not become denatured through heat coagulation. It is well known to the bacteriologist that coagulated proteins, such as cooked meats or boiled eggs, are more vulnerable to enzyme action, and bacteria find a more suitable substrate for growth on them than on raw substances. Hams and shoulders, when tenderized (partial cooking), or cooked in the smokehouse so that they are ready to eat, are to be considered in the same category as comminuted, cured domestic sausage products which must always be kept under adequate refrigeration. It has been shown (43) that cooked hams, cooked poultry and stuffings, sandwiches prepared with ham, eggs, fish, and fowl, and soup stocks should never be held in the "incubation zone" of staphylococci longer than four hours if gastrointestinal irritation due to staphylococci is to be prevented. The incubation zone is considered to be from 60 to 115 F (15.6 to 46.1 C), and as a safety margin for institutional and military mess halls the range from 50 to 120 F (10 to 52.9 C) is advocated.

(d) *Control of Spores in Canned Meats.* The heating schedules for canned meats developed through the stimulus of war have been based largely upon existing data derived from studies of spores. The direction of the work in the practical canning of spiced ham, luncheon meats, and sausage products is to retort the canned food according to a "botulinum schedule." Many meat foods subjected to this temperature would not be very palatable; hence, the need arose to produce a canned meat that would neither spoil nor present a health hazard. Also high-temperature processing for long periods lessens the vitamin content of meat foods. The thiamin and pantothenic acid content, especially, may be reduced, although niacin and riboflavin are not significantly reduced. When 3.5% NaCl, 2.33 oz. (0.15%) NaNO$_3$, and 0.125 oz (0.008%) NaNO$_2$ in each 100 pounds of comminuted meat are used, and the cured meat is packed in 6-pound cans and retorted for 3.3 hours so that the inside temperature reaches 225 F

(107.2 C), the processed cans of meat, even though seeded with spores of *Clostridium sporogenes* and of *Bacillus sp.*, will keep at any temperature below 132 F (55.6 C) for a time depending upon the rate of production of hydrogen from the chemical action of the contents upon the metallic can (hydrogen swells).

As much as 22% soya flour or "meat extender" can be added to comminuted meats and canned according to this schedule of curing and heating with the resulting product stable under expected temperatures (132F [55.6 C] and lower). Tests were conducted by Jensen and Hess (49, 50) who inoculated soya flour to contain over one million spores of gas-forming, anaerobic thermophiles per gram. Thirty 6-pound cans of comminuted luncheon meat (pork and beef) containing 22% of this soya flour were incubated for 9 months at 132 and 99 F (55.6 and 37.2 C) and found to be stable. The control cans without salt and nitrate swelled and burst in a few days at these incubation temperatures.

The "bombage" of canned meat foods stored without refrigeration in the tropics has been caused by viable spores of *Bacillus sp.* These spores are not inhibited from germination by 3.5% NaCl with sodium nitrate. These spores must be killed to produce a stable canned pork or luncheon meat. Spores of thermophiles may remain dormant in canned meat for long periods of time. Recently, Wilson and Shipp (111) examined bacteriologically a can of roasted veal, packed for the explorer Parry in 1824, and recovered six strains of sporing bacilli. The strains grew best at 37 C, all strains grew well at 55 C, and some grew at 60 C. The survival of spores in this can of veal in viable condition for over a century appears to have no parallel in bacteriological records.

REFERENCES

1. ADAMI, J. G. 1899 On latent infection and subinfection, and on the etiology of hemochromatosis and pernicious anemia. J. Am. Med. Assoc., 33, 1509–1514, 1572–1576.

2. ALLEN 1932 Commercial Organic Analyses. Fifth ed., 9, 275–276. P. Blakiston's Sons & Co., Philadelphia, Pa.

3. ARNOLD, L. 1928 The passage of living bacteria through the wall of the intestine and the influence of diet and climate upon intestinal auto-infection. Am. J. Hyg., 8, 604–632.

4. BARNARD, R. D., AND GOWEN, G. H. 1932 Greenish discoloration produced on blood agar by the growth of pneumococcus. Proc. Soc. Exptl. Biol. Med., 29, 521–524.

5. BITTENBENDER, W. A., DEGERING, E. F., TETRAULT, P. A., FEASLEY, C. F., AND GWYNN, B. H. 1940 Bactericidal properties of commercial antiseptics. Ind. Eng. Chem., 32, 996–998.

6. BLUM, H. F. 1941 Photodynamic action and diseases caused by light. Chap. 9. A.C.S. Monograph, New York, N. Y.

7. BOXER, S. 1906 Ueber das Verhalten von Streptokokken und Diplokokken auf Blutnährböden. Centr. Bakt., Parasitenk., Abt I, Orig., 40, 591–600.

8. BOYER, E. A. 1926 A contribution to the bacteriological study of ham souring. J. Agr. Research, 33, 761–768.

9. BROH-KAHN, R. H., AND MIRSKY, I. A. 1938 Studies on anaerobiosis. The nature of the inhibition of growth of cyanide-treated *E. coli* by reversible oxidation-reduction systems. J. Bact., 35, 455–475.

10. BROOKS, J., HAINES, R. B., MORAN, T., AND PACE, J. 1940 The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Dept. Sci. Ind. Research, Food Invest., Special Rept. 49, 2–4, London.

11. BURN, C. G. 1934 Postmortem bacteriology. J. Infectious Diseases, 54, 395–403; Experimental studies of postmortem bacterial invasion in animals. Ibid., 388–394.

12. CANFORA, M. 1908 Ueber die Latenz der Tetanussporen im tierischen Organismus. Centr. Bakt., Parasitenk., 45, 495–501.

13. CLAYTON, W., AND GIBBS, W. E. 1927 Examination for halophilic micro-organisms. Analyst, 52, 395–397.

14. DEAN, H. K. 1938 Utilization of Fats, 186–194. Chemical Publishing Co., New York City.

15. EWELL, A. W. 1940 The tenderizing of beef. J. Am. Soc. Refrig. Eng., April, 237–240.

16. EWELL, A. W. 1942 Production, concentration and decomposition of ozone by ultra-violet lamps. J. Applied Phys., 13, 759–767.

17. EWELL, A. W. 1942 Cutting shrinkage losses in retail meat coolers. The National Provisioner, April 11, 1942.

18. EWELL, A. W. 1942 Conservation of refrigeration during wartime. Refrig. Eng., September, 1942.

19. ESTY, J. R., AND MEYER, K. F. 1922 The heat resistance of the spores of *B. botulinus* and allied anaerobes. XI. J. Infectious Diseases, 31, 650–663.

20. FALK, I. S. 1923 The rôle of certain ions in bacterial physiology. Abstracts Bact., 7, 33–50.

21. GIBBONS, N. E., AND REED, G. B. 1930 The effect of autolysis in sterile tissues on subsequent bacterial decomposition. J. Bact., 19, 73–88.

22. GREEN, D. E., AND PAULI, R. 1943 The antibacterial action of the xanthine oxidase system. Proc. Soc. Exptl. Biol. Med., 54, 148–150.

23. GRINDLEY, H. S., MACNEAL, W. J., AND KERR, J. E. 1929 The influence of potassium nitrate on the action of bacteria and enzymes. Chap. 9, 2, 359. Studies in Nutrition, University of Illinois, Urbana, Ill. (Complete results in five volumes.)

24. HAGAN, W. A. 1925 The green coloration by certain streptococci on blood agar. J. Infectious Diseases, 37, 1–12.

25. HALDANE, J. 1901 The red colour of salted meat. J. Hyg., 1, 115–122.

26. HART, P. D'ARCY, AND ANDERSON, A. B. 1933 The formation of green pigment from haemoglobin by the pneumococcus. J. Path. Bact., 37, 91–105; the discolouration of heated blood agar by streptococci. Ibid., 334–335.
ANDERSON, A. B., AND HART, P. D'ARCY 1934 The viridans effect of the streptococci and the production of the green pigment from haemoglobin by other reducing systems. Ibid., 39, 465–479.

27. HEKTOEN, L., ROBSCHEIT-ROBBINS, F. S., AND WHIPPLE, G. H. 1928 The specific precipitin reaction of the muscle hemoglobin of the dog. J. Infectious Diseases, 42, 31–34.

28. HESS, E. 1942 Studies on salt fish, VIII. J. Fisheries Research Board Can., 6, (1), 1–23.

29. HOAGLAND, R. 1914 Coloring matter of raw and cooked salted meats. J. Agr. Research, 3, 211–226.

30. HOAGLAND, R., MCBRYDE, C. N., AND POWICK, W. C. 1917 Changes in fresh beef during cold storage above freezing. U. S. Dept. Agr. Bull 433.

31. HOLMAN, W. L. 1916 The classification of streptococci. J. Med. Research, 34, 377–387.

32. HOPPE-SEYLER 1871 Quoted from Wells, H. G., 1918. Chemical Pathology, 82. W. B. Saunders Co., Philadelphia, Pa.

33. HÜLPHERS, G. 1933–34 Skallvatten floran i Svinlungan. Nordiska Veterinärmötet, 800–813. Helsingfors, Finland.

34. INGRAM, M. 1939 The endogenous respiration of *Bacillus cereus*, II. J. Bact., 38, 613–629.

35. JACOBY, M. 1900 Ueber die fermentative Eiweisspaltung und Ammoniakbildung in der Leber. Z. physiol. Chem., 30, 149–174; 1901. Ueber autolyse der Lunge. Ibid.,

33, 126–130; 1903. Zur Frage der specifischen Wirkung der intracellularen Ferments. Beitr. Chem. Physiol. (Hofmeister's), 3, 446–450.

36. JENSEN, L. B. 1942 Microbiology of Meats, 18–24. The Garrard Press, Champaign, Ill.

37. JENSEN, L. B. 1942 *Ibid.*, 211–219.

38. JENSEN, L. B. 1942 *Ibid.*, 7–9; 155–163; 185–188.

39. JENSEN, L. B. 1942 *Ibid.*, Chapter 5.

40. JENSEN, L. B. 1942 *Ibid.*, 10, 17, 165.

41. JENSEN, L. B. 1942 *Ibid.*, 229.

42. JENSEN, L. B. 1943 Bacteriology of ice. Food Research, 8, 265–272.

43. JENSEN, L. B. 1944 Prevention of food poisoning by food preservation methods. J. Am. Vet. Med. Assoc., CIV, No. 802, 63–65.

44. JENSEN, L. B., AND GRETTIE, D. P. 1933 Action of microorganisms on fats. Oil and Soap, 10, 23–32.

45. JENSEN, L. B., AND GRETTIE, D. P. 1937 Action of microorganisms on fats. Food Research, 2, 97–120.

46. JENSEN, L. B., AND HESS, W. R. 1941 Action of nitrates on bacteria in cured meats. Food Manuf., 16, 157–167. London, England.

47. JENSEN, L. B., AND HESS, W. R. 1941 Fermentation in meat products by the genus *Bacillus*. Food Research, 6, 75–83.

48. JENSEN, L. B., AND HESS, W. R. 1941 A study of ham souring. Food Research, 6, 273–326.

49. JENSEN, L. B., AND HESS, W. R. 1943 Control of thermophilic bacteria in canned meat mixtures. Food Industries, 16, 66–68.

50. JENSEN, L. B., AND HESS, W. R. 1943 Comprobacion de la Bacteria Termofila en las Mezclas para Carnes Frias. La Maquina, 6, 16–19.

51. JENSEN, L. B., AND URBAIN, W. M. 1936 Bacteriology of green discoloration in meats and spectrophotometric characteristics of the pigments involved. Food Research, 1, 263–273.

52. KILMER, F. B. 1901 The story of the papaw. Am. J. Pharm., 73, 272–285.

53. KITASATO, S., UND WEYL, T. 1890 Zur Kenntniss der Anaeroben. Z. Hyg. Infektionskrankh., 9, 97–102.

54. KLUYVER, A. J., AND BAHRS, A. 1931 Konink. Akad. Wetenschappen, Amsterdam, Proc., 35, 370.

54. KOLLER, RAPHAEL 1938 Salt in the History and Culture of Mankind. *Hallein*, Austria.

56. KOSER, S. A., AND McCLELLAND, J. R. 1917 The fate of bacterial spores in the animal body. J. Med. Research, 37, 259–268.

57. KRAYBILL, H. R. 1943 Dehydration of meat. Ind. Eng. Chem., 35, 46–50.

58. LEA, C. H. 1938 Rancidity in edible fats. Dept. Sci. Ind. Research, Food Invest., Special Rep. No. 46 (London).

59. LEA, C. H. 1939 The deterioration of fats in foods. Chemistry & Industry, 58, 479–484, (London).

60. LEIFSON, E. 1931 Bacterial spores. J. Bact., 21, 331–356.

61. LEMBERG, R. 1935 Transformation of haemins into bile pigments. *Biochem. J.*, 29, 1322–1336.

62. LEMBERG, R., CORTIS-JONES, B., AND NORRIE, M. 1937 An oxyporphyrin haematin compound as intermediate between protohaematin and verdohaematin. Nature, 140, 65–66.

63. LEMBERG, R., CORTIS-JONES, B., AND NORRIE, M. 1938 Coupled oxidation of ascorbic acid and haemochromogens. Biochem. J., 32, 149–170; 171–186.

64. LEMBERG, R., AND LEGGE, J. W. 1943 Liver catalase. Biochem. J., 37, 117–127.

65. LEMBERG, R., AND LEGGE, J. W., AND LOCKWOON, W. H. 1939 Coupled oxidation of ascorbic acid and haemoglobin. *Biochem. J.*, 33, 754–758.

66. LEMBERG, R., AND LOCKWOON, W. H., AND LEGGE, J. W. 1941 Studies on the forma-

tion of bile pigments from choleglobin and verdohaemochromogen and on their isolation from erythrocytes. Biochem. J., 35, 363–379.

67. LEMBERG, R., AND WYNDHAM, R. A. 1937 Some observations on the occurrence of bile pigment hemochromogens in nature and on their formation from haematin and hemoglobin. J. Proc. Roy. Soc. N. S. Wales, 70, 343.

68. LINDSEY, G. A., AND RHINES, C. M. 1932 The production of hydroxylamine by the reduction of nitrates and nitrites by various pure cultures of bacteria. J. Bact., 24, 489–492.

69. LOCHHEAD, A. G. 1934 Bacteriological studies on the red discolorations of salted hides. Can. J. Research, 10, 275–286.

70. McCULLOCH, E. C. 1936 Disinfection and Sterilization, 491. Lea and Febiger, Philadelphia, Pa.

71. McLEOD, J. W., AND GORDON, J. 1922 Production of hydrogen peroxide by bacteria. Biochem. J., 16, 499–506.

72. McLEOD, J. W., AND GORDON, J. 1925 Further indirect evidence that anaerobes tend to produce peroxide in the presence of oxygen. J. Path. Bact., 28, 147–153.

73. MEYER, K. F. 1936 Latent infections. J. Bact., 31, 109–135.

74. MOULTON, C. R. 1926 Some factors affecting the water content of sausage. Am. Meat Inst., Chicago, Ill.

75. MOULTON, C. R., AND LEWIS, W. Lee, 1940. Meat Through the Microscope. Second ed., 250–256. University of Chicago, Chicago, Ill.

76. NIELL, J. M., AND HASTINGS, A. B. 1925 The influence of the tension of molecular oxygen upon certain oxidations of hemoglobin. J. Biol. Chem., 63, 479–492.

77. NORRIS, C., AND PAPPENHEIMER, A. M. 1905 A study of pneumococci and allied organisms in human mouths and lungs after death. J. Exptl. Med., 7, 450–472.

78. PETTERSON, A. 1899 Experimentelle Untersuchungen über das Conserviren von Fisch und Fleisch mit Salzen. Berlin. klin. Wochschr., 36, 915; 1900, Arch. Hyg., 37, 171–238.

79. QUASTEL, J. H., AND WOOLDRIDGE, W. R. 1927 The effects of chemical and physical changes in environment of resting bacteria. Biochem. J., 21, 148–168.

80. RAMSBOTTOM, J. M., AND RINEHART, C. A. 1940 Fruit enzymes. Food Industries, 12 (June), 45–47.

81. REEVES, J. R., AND MARTIN, H. E. 1936 The rôle of bacteria in autolyzing tissue. J. Bact., 31, 191–202.

82. REITH, A. F. 1926 Bacteria in the muscular tissues and blood of apparently normal animals. J. Bact., 12, 367–383.

83. RICHARDSON, W. D. 1907 Nitrates in vegetable foods, in cured meats and elsewhere. J. Am. Chem. Soc., 29, 1757–1767.

84. RITCHELL, E. C., PIRET, E. L., AND HALVORSON, H. O. 1943 Drying of meats: Rate of dehydration of uncooked cured ground meats. Ind. Eng. Chem., 35, 1189–1195.

85. ROBERTSON, MADGE E. 1931 A note on the cause of certain red colorations on salted hides, etc. . . . . J. Hyg., 31, 84–95.

86. ROCKWELL, G. E., AND EBERTZ, E. G. 1924 How salt preserves. J. Infectious Diseases, 35, 573–575.

87. RUEDIGER, G. F. 1906 The cause of green coloration of bacterial colonies in blood-agar plates. J. Infectious Diseases, 3, 663–665.

88. SALKOWSKI, E. 1890 Ueber Autodigestion der Organe. Z. klin. Med., 17, Supp. 77–100.

89. SHERMAN, J. M., AND ALBUS, W. R. 1924 The function of lag in bacterial cultures. J. Bact., 9, 303–305.

90. SMITH, F. B. 1938 The determination of halophilic vibrios (N. spp.) Proc. Roy. Soc. Queensland, Brisbane, 49, 29–52.

91. STEPHENSON, M. 1939 Bacterial Metabolism, 57. Longmans, Green and Co., New York, N. Y.

92. STUART, L. S., FREY, R. W., AND JAMES, L. H. 1933 U. S. Dept. Agr. Tech. Bull. 383.

93. STUART, L. S., AND SWENSON, T. L. 1934 Some new morphological and physiological observations on salt tolerant bacteria. J. Am. Leather Chem. Assoc., 28, 142–158.

94. TANNER, F. W. 1932 Microbiology of Foods, 482. Twin City Printing Co., Champaign, Ill.

95. TANNER, F. W., AND EVANS, F. L. 1933 Effect of meat curing solutions on anaerobic bacteria. Zentr. Bakt., Parasitenk., Abt. II, 88, 44–54.

96. TAROZZI, G. 1906 Ueber das Latentleben der Tetanussporen im tierischen Organismus und über die Möglichkeit das sie einen tetanischen Prozess unter dem Einfluss traumatischer und nekrotisierender Ursachen hervorrufen. Centr. Bakt., Abt. 1. orig., 40, 305–311; 451–458.

97. TARR, H. L. A. 1940 Specificity of triamineoxidease. J. Fisheries Research Board, Can., 5 (2), 187–196.

98. TARR, H. L. A. 1941 The bacteriostatic action of nitrites. Nature, 147, 417–418; The action of nitrites on bacteria. 1942 J. Fisheries Research Board Can., 6, 74–89.

99. THOMPSON, JAMES WESTFALL 1942 History of livestock raising in the United States, 1607–1860. U. S. Dept. Agr., Agr. History Series No. 5.

100. TOPLEY, W. C., AND WILSON, G. S. 1938 The Principles of Bacteriology and Immunity. Second ed. William Wood and Co., Baltimore, Md.

101. TRESSLER, D. K. 1920 Some considerations concerning the salting of fish. U. S. Bur. Fisheries Documents, 884, 1–55.

102. TRESSLER, D. K., AND MURRAY, W. T. 1932 How brining with pure salts improves fillets. Fishing Gaz., 49, No. 2, 1–3.

103. URBAIN, W. M., AND JENSEN, L. B. 1940 The heme pigments of cured meats. I. Preparation of nitric oxide hemoglobin and stability of the compound. Food Research, 5, 593–606.

104. VALENTINE, E. 1926 Differences in peroxide production and methemoglobin formation of green (alpha) streptococci. J. Infectious Diseases, 39, 29–47.

105. VAN BRUGGEN, J. T., REITHEL, F. J., CAIN, C. K., KATZMAN, P. A., AND DOISY, E. A. 1943 Penicillin B: Preparation, purification, and mode of action. J. Biol. Chem., 148, 365–378.

106. VILJOEN, J. A. 1926 Heat resistance studies. 2. The protective effect of sodium chloride on bacterial spores heated in pea liquor. J. Infectious Diseases, 39, 286–290.

107. VON FÜRTH, OTTO 1916 Physiological and Pathological Chemistry of Metabolism, 77. J. B. Lippincott Co., Philadelphia, Pa.

108. VON LOESECKE, H. W. 1943 Drying and Dehydration of Foods. Reinhold Publishing Corp., New York, N. Y.

109. WELLS, H. G. 1925 Chemical Pathology. Fifth ed., Chap. III. W. B. Saunders Co., Philadelphia, Pa.

110. WILSON, J. A. 1928 The Chemistry of Leather Manufacture, 165–166. Chemical Catalog Co., New York, N. Y.

111. WILSON, G. S., AND SHIPP, H. L. 1939 Historic Tin Foods. Intern. Tin Research Development Council, Publication 85, 49–55. Fraser Road, Greenford, Middlesex, England.

112. WINSLOW, C.-E. A., AND FALK, I. S. 1923 Studies on salt action, VIII, IX. J. Bact., 8, 215–236, 237–244.

113. YESAIR, J. 1930 Canning Trade, 52, 112–115; Bull. Nat. Canners' Assoc., Washington, D. C.

114. YESAIR, J., AND CAMERON, E. J. 1942 Effect of curing agents on anaerobic spores. National Canners' Association. Washington, D. C.

115. ZINSSER, H., AND BAYNE-JONES, S. 1939 Textbook of Bacteriology. Eighth ed., 317, 934. D. Appleton-Century Co., New York City.

116. ZOBELL, C. E. 1932 Factors influencing the reduction of nitrates and nitrites by bacteria in semisolid media. J. Bact., 24, 273–281.

117. ZOBELL, C. E., ANDERSON, D. Q., AND SMITH, W. W. 1937 The bacteriostatic and bactericidal action of Great Salt Lake water. J. Bact., 33, 253–262.

THE PARASITIC ACTINOMYCETES AND OTHER FILAMENTOUS MICROÖRGANISMS OF THE MOUTH

A Review of Their Characteristics and Relationships, of the Bacteriology of Actinomycosis, and of Salivary Calculus in Man

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CONTENTS

This review, one of a series on the bacteriology of the human mouth (113, 114), deals with a group of gram-positive, filamentous microörganisms, both branched and unbranched, which are cultivable under partial anaerobiosis or in the presence of carbon dioxide. The group comprises the branched genus *Actinomyces* and the unbranched genus *Leptotrichia*, which are dealt with in detail; while certain other microörganisms, to which the generic names *Actinomyces* and *Leptotrichia* (or "*Leptothrix*") have been applied, are discussed in an effort to clarify their hitherto confused relationships. The etiology and pathogenesis of actinomycosis in man, and the nature and manner of formation of salivary calculus or tartar, are considered with special reference to these microörganisms.

THE PARASITIC ACTINOMYCETES[1]

The actinomycetes are gram-positive microörganisms characterized by the formation of a mycelium or network of branched and rebranched filaments. Mycelium formation is a property of the true fungi, and the actinomycetes are often classed with the fungi rather than with the bacteria proper. The

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[1] For the purposes of this discussion, the terms "parasitic actinomycete" and "actinomyces" are employed interchangeably as common names for organisms of the genus *Actinomyces*. The common name "actinomycete" is employed here in its customary inclusive sense. The terms "parasitic" and "saprophytic" are employed in the following sense: "parasitic" = living on or in another organism and deriving nourishment therefrom; "saprophytic" = living on dead organic matter. It may be noted that neither of these terms implies pathogenicity. Many parasitic microörganisms produce disease, but some, e.g., the white staphylococci of the skin or the pigmented *Neisseria spp.* of the throat, are not known to do so. Conversely, most saprophytes lack disease-producing capacity, but the clostridia of gas gangrene are a notable exception.

group, however, shows a wide range of variation within itself, and the members of it with which this review is concerned are clearly much more bacteria-like than fungus-like. On the whole, the actinomycetes may best be considered as intermediate between the bacteria proper and the true fungi, or, in the words of Waksman (143), as "an independent group of organisms which is closely related to the bacteria through some of the constituent forms, but which has adopted a fungus-like form of growth."

This review deals with only one subdivision of the actinomycetes, those of strictly parasitic habit, which are included here under a single specific name, *Actinomyces israeli*. The natural habitat of these organisms appears to be the mouth and throat. They are not found in nature apart from the tissues of man and animals, or otherwise under saprophytic conditions, and their properties are such as to indicate their incapacity to multiply or even to survive under such conditions. They appear, in other words, to be obligate parasites. They seem ordinarily to be harmless, but under exceptional circumstances they give rise to actinomycosis, a disease of which they are the principal cause both in man and in animals. These parasitic actinomycetes should be distinguished sharply from the broad and varied category of *saprophytic actinomycetes*, with which this paper is concerned only for incidental and comparative purposes (see table 1). The saprophytic organisms inhabit the soil, and are widely distributed on grains and grasses. This group includes both pathogenic and non-pathogenic members. It may be emphasized that the relationship between the saprophytic and the parasitic actinomycetes depends on two points of resemblance only. Both are composed of a gram-positive, branched mycelium, and both may be pathogenic for man and animals and produce lesions in which radially clubbed "sulfur granules" are found. The saprophytic forms, on the other hand, are recovered only rarely from true actinomycosis ("lumpy jaw") in cattle, and their association with true actinomycosis in man seems to be even more unusual. They are found, however, in certain tropical skin diseases ("mycetomas") of both man and animals.

The generic name *Actinomyces* ("ray fungus") was originally given by Harz (56) to organisms observed in material from lumpy jaw in cattle. It was Harz likewise who applied the name actinomycosis to this disease. These terms have come to be used nearly universally for the disease and for its most common causative agent, the parasitic actinomycete. Unfortunately the term *Actinomyces* has also been used for quite distinct members of the saprophytic group. This confusing double usage will be resolved if the recent recommendation of Waksman and Henrici (145) achieves general acceptance. After several attempts had been made to distinguish the saprophytic and parasitic groups by reserving the generic name *Actinomyces* for the former and using for the latter such names as *Cohnistreptothrix* (107, 143) or *Actinobacterium* (110), Waksman and Henrici (145) have now applied the generic term *Actinomyces* exclusively to the parasitic organisms with which this review deals, while the genus of aerobic non-sporebearing forms, formerly called *Proactinomyces*, is now *Nocardia*, and the genus of aerobic forms with spores in chains on aerial hyphae, previously designated *Actinomyces*, is now *Streptomyces*.

The full name *Actinomyces israeli*, applied to the parasitic forms, accords well with international usage. The specific name is credited to Kruse by the Argentine workers, Negroni and Bonfiglioli (102), who have adopted it, as

TABLE 1

Comparison of the Parasitic and Saprophytic Actinomycetes

	PARASITIC ACTINOMYCETES	SAPROPHYTIC ACTINOMYCETES*
Natural habitat	Mouth and throat of man and probably of cattle and other animals; obligate parasites; sometimes pathogenic.	Soil, grains and grasses; widely distributed in nature; some pathogenic species, but most forms are non-pathogenic.
Cellular morphology	Branched mycelium, gram-positive, not acid-fast. Marked tendency to fragment into bacillary forms.	Branched mycelium, gram-positive; some are acid-fast. Generally little tendency to fragment into bacillary forms.
Character of growth	Bacteria-like colonies without aerial hyphae; no spores; no pigments.	Colonies more mold-like, often with aerial hyphae and spores (conidia); many produce yellow, orange or black pigments.
Temperature requirements	Optimum, 37 C; no growth at 22 C.	Optimum usually 15–20 C.
Relation to oxygen	Oxygen tolerance limited; generally fail to grow or grow poorly under aerobic conditions.	Aerobic; some forms do not grow anaerobically.
Metabolism	Probably never proteolytic. Ferment carbohydrates with production of acid.	Many forms actively proteolytic; may utilize carbohydrates without acid production.
Species recognized	One only: *Actinomyces israeli.* (Provisional; heterogeneous but not yet satisfactorily subdivided.)	Many, subdivided into several families.
Pathogenicity	Causative agent of true actinomycosis in man and animals.	Occasional causes of an actinomycosis-like disease, very rare in man, and of tropical cutaneous mycetomas, e.g., Madura disease.

* Further details of the saprophytic actinomycetes are given by Henrici (58), St. John-Brooks (120), Erikson (42), and Waksman (143).

has Grootten (50) in France, Puntoni (110) in Italy, and Waksman (143) in this country. It seems best to discard the names *Actinomyces bovis* and *A. hominis*, since both have been used so loosely as to require the modifying phrase, "Wolff-Israel type" to make them apply without ambiguity to the parasitic

form. The name *Actinomyces israeli* is appropriate in view of the fact that Israel (61) was the first to recognize its parasitic nature, and since Wolff and Israel (152) first cultivated it from actinomycosis and laid the foundation for our present knowledge of the etiology and pathogenesis of that disease.

Actinomyces israeli

Parasitic actinomycetes with the characteristics described below have been isolated from actinomycosis in man, cattle and other animals by Wolff and Israel (152), Wright (153), Colebrook (21, 22), Lignières (81) and many others. Wright predicted in 1905 that they would be found as a normal inhabitant of the mouth. Lord (84, 85) demonstrated them in sections of carious detritus and in material from tonsils; and they have since been isolated from the human mouth and throat by many workers (97, 99, 86, 41, 130, 126), who have shown these organisms to be very similar to or indistinguishable from those of actinomycosis.

Morphology. *A. israeli* is a gram-positive, branching, filamentous organism. It is not acid-fast, does not form spores, and is non-motile. The individual filaments are generally less than 1 micron in width, like most bacteria, and unlike the true fungi, which are usually several times as wide. *A. israeli*, however, varies markedly in appearance under different conditions. Since some of the difficulties in this field seem to have been determined in the past by failure to recognize or to identify this organism, a full description of it seems warranted here.

In tissue sections made from the lesions of actinomycosis, the organism appears in the form of compact granules or colonies which are often visible to the unaided eye. The characteristics of the granule are frequently clear enough for diagnostic purposes under low magnification (50 to 100 diameters), but their details are best seen under higher power (400 diameters or more). The granule may be roughly circular or irregular in outline, or may be seen to consist of several colonies of different size and shape which have coalesced. Each granule is composed of a dense reticulum of fibrils which may stain irregularly in hematoxylin-eosin preparations but take the violet dye in sections stained by Gram's method. Around the periphery of the granule the ends of individual filaments may be seen projecting, or, more characteristically, part or all of the periphery may be composed of the radially arranged hyaline *clubs* to which the actinomycetes owe their name. The clubs take the eosin stain; they are several times wider than the filaments whose ends they enclose, and the filament can sometimes be traced within the structure of the club. Such radial clubs are often regarded as primary diagnostic features of the true actinomycotic granule, but their value for the purpose is only relative, since granules composed of *A. israeli* may fail to show clubs, and, as noted below, similar if not identical clubs may be found in other disease processes, notably actinobacillosis.

In exudates from actinomycosis, colony particles are frequently but not always present, and may be macroscopically visible as *sulfur granules*,—irregularly spherical or mulberry-like masses, usually whitish or pale yellow, from a fraction

of a millimeter to about 2 mm in diameter. The granules are usually soft and easily broken under light pressure, but they may occasionally be tough or even calcified. In a wet, unstained slide-coverslip preparation the crushed granule appears as a more or less disorganized mass of irregular, bent and branching filaments, some of which may terminate in the characteristic clubs. In preparations fixed and stained by Gram's method the structure of the granule is usually completely lost; clubs are not in evidence, and although some individual branching filaments may appear, the typical picture is that of a mass of irregular, bent gram-positive rods with projecting portions which give the fragments a *twig-like* appearance.

In preparations from the mouth or throat in the absence of actinomycosis, these organisms may take several forms. Clubs are not seen, and clearly branched filaments are seldom apparent. Lord (85) has described the occurrence of branching filaments in tissue sections of scrapings from carious teeth and of masses taken from tonsillar crypts; and Naeslund (99), Soderlund (128) and others have described a similar picture, with occasional clubs, in sections of salivary calculus. Clumps of bent twig-like rods, usually with little or no clear evidence of dendritic branching, can often be seen in stained films made from these locations, or from gingival scrapings from a pyorrheal pocket. That such organisms are in fact *A. israeli* is indicated by their recovery in cultures from such sources; but microscopic examination is seldom sufficient in itself to justify their identification. Darkfield examination of wet preparations is an excellent method for this purpose, since the clumps retain their character more perfectly in wet preparations than when dried for staining, and the darkfield method shows the continuity of structure of the twig-like and branched forms. On the other hand, evidence from culture preparations suggests that *A. israeli* may also occur on the oral and pharyngeal mucous membranes as unbranched gram-positive rods or diphtheroid forms which would defy identification by means of the microscope alone.

In cultures, the morphology of *A. israeli* runs the gamut from a compact mass clearly composed of a branching mycelium of gram-positive filaments to a quite undistinguished picture of regular short rods which may be evenly stained or granular, and which show no indication of branching. These differences are associated with roughness or smoothness of colony form. Rough colonies, whether they grow on an agar surface, in the depths of an agar shake culture or in broth, show branching forms regularly when prepared for microscopic examination with care to avoid disorganization of the mass. Broken twig-like forms, however, are much more common than long filaments. Intermediate and smooth colonies often yield a picture that resembles that of the diphtheria bacillus, with granular and polar-stained forms disposed in V- and Y-groupings, and with suggestive but not conclusive evidence of branching. Some smooth colonies, derived after repeated subculture from rough and clearly branched forms, may appear as short evenly stained rods with no distinguishing characteristics. The rough and intermediate forms often show terminal swellings or "clubbed forms" like those of the diphtheria bacillus; but the true clubs

seen in sections of actinomycotic tissue do not appear in either wet or fixed microscopic preparations made from cultures of *A. israeli*. Wright (153) obtained clubs irregularly in cultures by growth or persistence in the presence of high concentrations of serum or ascitic fluid. Lord and Trevett (86) were unable to produce clubs by similar means. It has been generally assumed that club formation represents a response of the tissues to the presence of the organism. Bayne-Jones (4), on the other hand, obtained typical eosin-staining clubs in sections of a colony from a glucose broth culture.

Growth and Metabolism. The isolation and maintenance of pure cultures of *A. israeli* have often been found difficult, apparently only because two characteristics of the organism have not been widely recognized:

1. Strains are apt to die out if cultivated successively on any single medium, but generally thrive if transferred alternately to different media (43, 115).

Among the media recommended, in addition to infusion broth or agar containing 1 per cent of glucose, have been Dorset's egg or glycerin-egg medium (43), Lubinski's medium (102), chopped-meat infusion broth, and Bacto brain-heart infusion to which 2 per cent of agar is added (115). None of these media appears to be fully satisfactory, in itself; hence the need for alternate transfers, apparently on the "varied diet" principle.

2. The organism has a *limited tolerance for oxygen* which varies from strain to strain and in individual strains at different times. Some strains grow only under anaerobic conditions, while others may grow aerobically, especially in broth; but any strain is likely to be lost if maintained through successive transfers under aerobic conditions only. Rosebury, Epps and Clark (115) were able to maintain 15 strains without difficulty by cultivation in anaerobic jars containing 5 per cent of carbon dioxide, which was found to favor continued growth.

A. israeli grows best at 37°C and fails to grow at 22°C. The optimum pH range for growth is 7.2 to 7.6 (21, 101). According to Negroni and Bonfiglioli (102) no growth occurs in the absence of carbohydrate; but other workers have obtained growth in plain nutrient broth or agar, and the writer has cultivated the organism successfully in fluid media negative to Benedict's reagent. Growth is much more abundant, however, in the presence of glucose.

Acid without gas is produced from a wide range of carbohydrates (81, 60, 102, 43). Naeslund (97) stated that some strains showed definite proteolytic action on coagulated egg albumin and serum and on gelatin, in the presence of sterilized saliva and glucose; but other workers have found *A. israeli* uniformly non-proteolytic (81, 86, 102, 43). Lignières (81) reported no production of indole, while Negroni and Bonfiglioli (102) found indole produced in small amounts, and also recorded reduction of nitrate with two strains but not with others. According to these workers, H_2S is formed in glucose agar containing liver extract and lead acetate; and washed human red blood cells are hemolyzed, but not rabbit cells or whole blood. Most workers have found *A. israeli* non-hemolytic. Pigments or antimicrobial substances such as have been obtained from species of saprophytic actinomycetes (see Waksman, 144) have not been recovered from this organism.

A. israeli is easily killed by moderate heating: in 3 to 10 minutes at 62 to 64°C (153); in 1 hour at 60°C (21); or in 30 minutes at 60 to 65°C (102). Wright (153) reported that granules in actinomycotic pus were still viable after being dried in test tubes in air for 18 to 22 days. Cultures dried over anhydrous phosphoric acid in vacuum by Negroni and Bonfiglioli (102) remained alive for 3 or 4 months in the ice chest. Lyophilization or similar methods of preservation do not seem to have been tried with this organism. Freshly isolated cultures in agar shake tubes, kept in the refrigerator, should be transferred every two weeks, while older strains may remain viable in this medium in the cold for about a month.

Cultural Characteristics. The appearance of vigorously growing *A. israeli*, as noted above, varies in relation to the roughness or smoothness of the strain. When first isolated from parasitic or pathological sources in man most if not all strains are either rough or intermediate. Some strains show little tendency to change, but others become smooth after prolonged artificial cultivation. The rough strains are the more distinctive.

In glucose-agar shake cultures incubated in air at 37°C, growth is seldom visible within 48 hours and more commonly requires from 3 to 6 days. The colonies are white or greyish, roughly spherical or compound, "crumb-like" and up to about 1.5 mm in diameter. The appearance of rough strains in this medium is generally highly characteristic and hence of diagnostic value; growth is concentrated in a zone about 5 mm wide, with its upper limit from 0.5 to 2 cm below the free surface. Scattered colonies may be present below and occasionally above this zone, but growth does not occur on the exposed surface. With smooth strains there may be no zone of concentrated growth, but uniformly distributed colonies may extend from the bottom of the tube to a level 0.5 to 1 cm from the surface, at which the growth terminates abruptly. When a whole colony of a rough strain in this medium is transferred with a capillary pipette to a slide, the colony is often found to be tough and difficult to break up and emulsify; but either darkfield or gram-stained preparations show the characteristic compact branched mycelium.

In glucose broth, incubation in air at 37°C may be successful through one or more subcultures; but anaerobic incubation is more reliable. In either instance the growth of rough strains is again very characteristic: they appear as white or greyish masses, up to about 5 mm in diameter, at the bottom of the tube, the medium itself remaining perfectly clear. Colebrook (21) has aptly described these masses as resembling tiny cauliflowers or breadcrumbs. Like other colonies of rough strains they are often difficult to break up. Transfer of the supernatant medium to a fresh culture medium fails to secure growth; it is necessary to transfer a visible fragment of the colony. Intermediate strains tend to grow as smaller particles or granules either at the bottom or along the side of the tube, or as viscid or flocculent masses, again with little or no general turbidity; while smooth strains, particularly under anaerobic conditions, may produce uniform turbidity with or without a viscid or granular sediment.

Surface colonies on suitable media, such as glucose agar or brain-heart agar, incubated anaerobically with 5 per cent CO_2 for 4 to 6 days, are sufficiently

distinctive with rough or intermediate strains to make this the method of choice for the isolation of *A. israeli*, particularly from contaminated sources. On sparsely seeded plates the colonies may have a diameter as great as 3 mm, but more commonly they are 1 mm in diameter or less. To the naked eye they appear dead white, or more rarely slightly greyish or yellowish. They are opaque, matt, and somewhat pitted or irregular in both surface and outline. Under a magnification of 5 to 10 diameters, and with suitably reflected illumination, rough colonies present a glistening but irregular surface and a high-raised contour, "heaped-up" in Erikson's (43) phrase, somewhat like colonies of the tubercle bacillus, but on a smaller scale. Such colonies are usually found to be strongly adherent to the medium, so that they are hard to remove with an inoculating needle, often come away all in one piece, and are emulsified with difficulty. Intermediate colonies may have a smooth but irregularly contoured surface which, with their white color and peculiar opacity, may give them a striking resemblance to the crown of a human molar tooth, as noted by Sullivan and Goldsworthy (130). Completely smooth colonies, on the other hand, are not distinctive, but resemble the smooth round raised colonies of white staphylococci or diphtheroids. They are soft and easily broken and emulsified. Whether such smooth actinomyces colonies ever appear on primary plates from contaminated sources is not known; if they do it would not be easy to mark them apart from other colonies on the plate, and it would be particularly difficult to distinguish them from certain diphtheroids. It may be noted that some of the common aerobic bacteria, particularly streptococci, may appear on anaerobic-CO_2 plates in colonies very different from the familiar ones on aerobic media; and an occasional rough white colony similar to those described above may turn out on examination to be a streptococcus.

Subdivision of A. israeli. Several attempts have been made to subdivide the parasitic actinomycetes into distinct groups, but none of the proposed classifications has been generally accepted, perhaps because the number of strains studied by any one investigator has never been large enough to make reported differences seem convincing. The bases for subdivision have been differences in (a) oxygen tolerance (97); (b) morphology and growth rate (41); (c) fermentation reactions and agglutination (60); and (d) colony morphology, correlated with source of strains and with agglutination reactions (43, 78). Naeslund (97) and Emmons (41) recognized that their groups were closely interrelated. Holm's (60) two groups seemed to be distinct, but he studied only 9 strains, all derived from actinomycosis in man. Lentze (78) and Erikson (43) found independently that fermentation reactions were too variable to be useful for classifying strains derived from actinomycosis in both man and cattle. These workers reported that bovine strains were usually smoother than human strains and that they were distinct by agglutination. Erikson, moreover, found 5 bovine strains to be more tolerant of oxygen than her 15 strains from human disease, and also less exacting in their need for CO_2. Strains of *A. israeli* isolated from mucous membranes of persons without actinomycosis were not included in these studies. Such strains seem to be smooth more often

than those obtained from actinomycosis in man (115). Smoothness or roughness is, however, a dubious basis in itself for classification. It appears on the whole that *A. israeli* is a heterogeneous species; but until more extensive data are available it seems unwise to attempt to subdivide it. Data given below show that strains from all three common sources, actinomycosis in man and in cattle, and non-actinomycotic mucous membranes, may all be capable of producing actinomycosis under experimental conditions.

Interrelationships of A. israeli with Other Microörganisms

Taxonomic Position of the Actinomycetes. The whole order *Actinomycetales* exemplifies, perhaps as well as any biological group, the continuity of the living world, and the fluidity of the boundaries which are drawn in an effort to separate it into larger or smaller categories. Sometimes called "higher bacteria," they seem to be related through the diphtheria bacillus, the tubercle bacillus, and the closer relatives of both, to the bacteria proper; and through the true fungi to the plant kingdom as a whole. Because of their apparent intermediate position the writer has refrained from speaking of them either as bacteria or as fungi.

Actinomyces and Lactobacilli. The parasitic actinomycetes may have an additional bond of relationship to the bacteria proper, through lactobacilli of the bifidus type (114). The lactobacillus isolated from the intestines of guinea pigs by Crecelius and Rettger (27) seems particularly reminiscent of an actinomycete intermediate between the rough and smooth forms described above, in its morphology, its nonfastidious anaerobic habit, and in other respects. The only direct comparison between *A. israeli* and *Lactobacillus bifidus* that seems to have been made is that of Puntoni (109), who reported that differences between the two groups in morphology and in biochemical and serological characteristics were quantitative rather than qualitative and lay within the range of expected variation of the two heterogeneous groups. This subject seems worth further study.

Parasitic and Saprophytic Actinomycetes. The parasitic actinomycetes are ordinarily so clearly distinct from the saprophytic forms that there would seem to be little reason to confuse them. Two points of difficulty have nevertheless arisen, as follows:

1. Members of the *saprophytic* group have been observed in and isolated from actinomycosis, especially in cattle (14, 81) and they have also been cultivated from the human mouth (97, 125). Naeslund (100), moreover, has found that rare strains of saprophytic actinomycetes—two strains out of several hundred isolated from plants and soil—were capable of producing actinomycosis-like disease in experimental animals. None of 30 saprophytic strains isolated from mucous membranes of human subjects without actinomycosis had this property. Two other instances are cited by Colebrook (22) and a third by Wright (154) in which saprophytic actinomycetes seem to have been responsible for suppurative lesions containing typical granules. In an earlier study Naeslund (97) had been able to recover *A. israeli* from the human mouth in greater

abundance than the saprophytic forms; and in view of the greater resistance and widespread distribution in nature of these latter, he argued that they are chance invaders and not indigenous to the mouth. He noted that 3 out of 30 saprophytic strains isolated from the mouth grew poorly at 37°C but luxuriantly at 15 to 20°C,—a clear indication that these strains, at all events, were not indigenous.

According to the data collected by Colebrook (22) the truly aerobic (saprophytic) actinomycetes have been recovered in only a small minority of instances from actinomycosis in cattle, and rarely if ever from the disease in man (1 doubtful case out of 94). Cope (25) notes that the occasional recovery of saprophytic actinomycetes from lesions in man may be due to contamination, but believes that they may at times cause such disease. Biggart (12) has recorded a case of generalized and rapidly fatal disease following hysterectomy, from which a true saprophytic actinomycete was recovered. The organism produced small non-fatal lesions in experimental animals. No granules were found either in the patient at autopsy or in the animals inoculated with cultures.

The facts thus do not justify categorical exclusion of the saprophytic actinomycetes as incitants of actinomycosis; but it seems clear that their importance in the human disease is of a very low order.

2. The use of the term *anaerobic actinomyces* as an overall designation for the parasitic forms (e.g., Colebrook, 23) seems to have led to confusion in that some strains whose characteristics place them with *A. israeli* have not been classified as such because they were found able in some degree to grow in air. Anaerobiosis within the parasitic group is relative and variable, and its variation does not afford a basis for their subdivision. The group as a whole is easily distinguished from the aerobic saprophytic group by other characteristics (table 1). Organisms which seem to have been *A. israeli* but were able to grow in air, and which were regarded as ambiguous either by the original author or by others, have been described by Naeslund (97), Lord and Trevett (86), Bibby and Knighton (11), Crowley (28), and Bartels (3).

The relationship to actinomyces of organisms described under this and other names, including *Leptothrix* and *Cladothrix*, is discussed below.

THE LEPTOTRICHIA

The genus *Leptotrichia* comprises certain *unbranched* gram-positive rods and filaments which occur characteristically and prominently as parasites of the human mouth, but which have no known pathogenic or other significance. Only a single species, *Leptotrichia buccalis*, can be defined with any assurance. The names *Leptotrichia* and *Leptothrix* have however been used widely and very loosely to apply to a variety of forms, some of which can be placed with little doubt in distinct genera, while others have remained unidentified.

The name *Leptothrix* is not properly applied to this group. This term was first given by Kützing in 1843 (76) to a genus of filamentous iron bacteria which are free living and autotrophic, and cannot be considered even distantly related to the parasitic filaments under discussion. These parasitic forms were

first described as *Leptothrix buccalis* by Robin (111, 112). Similar microör-
ganisms had been described and pictured by Leeuwenhoek in his letter of 1683
(36) and by Buehlmann in 1840 (17). Trevisan, in 1879 (139), recognized the
distinction between Kützing's iron forms and those of the mouth, and proposed
the generic name *Leptotrichia* for the latter.

Leptotrichia buccalis is a gram-positive unbranched filamentous organism,
non-motile, non-sporulating, with a tendency to grow as coarse, often granular
threads or long rods which may be rounded and sometimes clubbed at one end
and tapered or pointed at the other. The most complete study of this organism
is that of Thjøtta, Hartmann and Bøe (137). Clear descriptions of it have also
been given by Kligler (72), who called it *Leptothrix buccalis*, and by Wherry
and Oliver (149), who gave it the name *Leptothrix innominata* as used earlier
by Miller (95).

According to Thjøtta and his collaborators this organism can always be seen
in smears from the mouth, especially from the central part of the dorsum of
the tongue. They succeeded in growing it on Bacto brain-heart infusion
medium containing up to 2 per cent of agar, and found that it grew better
"in a jar emptied of air and filled up with CO_2" than under either aerobic or
completely anaerobic conditions. The previous workers had described it as a
facultative anaerobe or microaerophile. Colonies are about 1 mm in diameter
after 24 hours, but continue to grow and reach a much greater size after 7 days'
incubation at 37°C. They are described as having a characteristic thatched or
medusa-head appearance and a pearly translucency and lustre, and are found
by microscopic examination to consist of "long streamers of rods or filaments
closely packed together like rafts of timber in a river." On blood agar the
colonies are smaller and more irregular; they are not hemolytic but may pro-
duce slight greening. Although distinctly gram-positive in young cultures
they become increasingly gram-negative later, and often show gram-positive
granules in a gram-negative base. They stain yellow with iodine. All strains
have been found to ferment several carbohydrates including starch, and to
acidify milk with or without coagulation. Final pH values ranged from 4.7
to 5.2. Gelatin was not liquefied, and neither indole nor H_2S was produced;
but some strains reduced nitrates. Complement fixation tests with 5 rabbit
antiserums showed ten strains to be mutually interrelated but not homogeneous;
while a strain of oral *Lactobacillus acidophilus* did not react with any of the
serums (137).

Kligler (72) believed this organism to be one of three that constitute the
essential flora of early dental caries, the others being *Cladothrix placoides* (see
below) and *L. acidophilus*. So far as this view relates to *Leptotrichia buccalis*,
it was based chiefly on the abundance of the organism in deposits from early
carious lesions and on its filamentous character, which suggested a capacity to
help fix the more essential decalcifying organisms to the tooth surface. *L.
buccalis* was found to be less active in acid production and decalcification than
either of the other forms, and did not augment their powers in these respects
when grown in combination with either or both. Kligler's view with regard

to this organism has not received confirmation, and there seems to be little reason at present to credit it.

Leptotrichia have also been associated with salivary calculus, as noted below; but descriptions under this name, or under the name *Leptothrix*, of organisms isolated from tartar have never conformed clearly with that given above. In some instances, notably the *Leptothrix buccalis* of Bulleid (18, 19), the forms isolated from tartar can be identified with little doubt as *Actinomyces israeli*. Otherwise the identity of organisms isolated from tartar and given the names *Leptotrichia* or *Leptothrix* remains uncertain. This statement applies even to the work of so careful a student of the actinomycetes as Naeslund (99). This author's description of "leptothrix" isolated from tartar fails to identify it clearly, although it seems probable that the organism was *not* an actinomycete, particularly since typical *A. israeli* was also described as having been recovered from the same source. The filamentous organisms isolated from tartar and other oral sources by Bibby and his co-workers (8, 9, 11) are also inadequately identifiable. Some of these seem to have been *A. israeli*, but to have represented a selection of more oxygen-tolerant strains; while others, described as branching leptotrichia, cannot be placed with any assurance in either group. Organisms isolated both from tartar and from excised gingival tissue by Grythe (52, 53, 54) seem likewise to have included *A. israeli* and possibly saprophytic actinomycetes as well; but other forms described as "leptothrix" are of doubtful identity. There seems consequently to be no evidence that true *Leptotrichia buccalis* occurs in salivary calculus, and no reason, despite the frequent use of this and similar names in relation to these concretions, to assign any rôle to this organism in its deposition.

Other attempts have been made to impute pathogenic significance to the oral leptotrichia, but without convincing evidence (see MacKenzie, 88A).

MISCELLANEOUS FILAMENTOUS MICROÖRGANISMS

Several apparently unrelated groups of microörganisms have been either (a) described under other names although they may belong with the parasitic actinomyces, or (b) described as actinomycetes or leptotrichia although they seem to belong in neither group.

Possible Actinomycetes Described Under Other Names. As noted above, organisms described as *Leptotrichia buccalis*, or loosely as leptothrix or leptotrichia, have sometimes been identifiable as *Actinomyces israeli*.

Leptotrichia placoides (Bergey et al., 5) may include either *A. israeli* or a rough lactobacillus, or possibly a form intermediate between the two; while some of the properties attributed to it seem to exclude it from both these groups. Dobrzyniecki (37, 38) first applied the name *Leptothrix placoides alba* to a gram-positive aerobic organism isolated from a root canal. It grew in chains of rods and tangled threads, failed to form spores, liquefied gelatin and blood serum, and grew at 16 to 18°C as well as at 37°C. Kligler (72) cultivated 58 strains which he believed to correspond with Dobrzyniecki's organism, and named it *Cladothrix placoides*. Kligler's strains were not uniform. Some were

gram-negative, and some liquefied gelatin. Most of them, however, were gram-positive and non-proteolytic. They fermented glucose, sucrose and in some instances lactose; reduced nitrates, and failed to form ammonia or indole. Morphologically they were variable, with coccoid, diphtheroid and filamentous forms, some of which showed swollen ends and "false" branching. The preponderant gram-positive non-liquefying form may have been an actinomycete which was not observed under conditions that permitted demonstration of true branching, although Kligler's statement that this form was aerobic and grew slowly at 20°C makes it doubtful that the organism was *A. israeli*. Bibby and Knighton (11) considered Kligler's organism to be the same as forms described by them, which in turn seem to have been selected aerobic strains of *A. israeli*; these organisms were non-proteolytic, but also grew slowly at room temperature.

Kligler regarded his *Cladothrix placoides* as second in importance to *Lactobacillus acidophilus* in the flora of early dental caries. This view was based in part on the common occurrence of the organism in the disease, along with lactobacilli and *Leptotrichia buccalis*; and also on the finding that *Cladothrix placoides* was second to *Lactobacillus acidophilus* in acidogenic power, all other species tested having been much less active. It is this finding which suggests that *C. placoides* may have been a rough lactobacillus; and there is nothing in Kligler's description of the majority of his strains that would definitely contra-indicate this view.

Other So-called Leptothrices and Actinomyces. The names *Leptothrix* and *Actinomyces* have been applied rather indiscriminately to several additional microorganisms, not all of which can be identified from published descriptions, but none of which appear to fit into either of these genera.

As early as 1890, Miller (95) remarked that the name *Leptothrix buccalis* had been applied loosely, and this has continued to be true. Miller's own use of this generic term is confusing. His *Leptothrix buccalis maxima* and *Leptothrix innominata* may both have been true *Leptotrichia buccalis*. His *Bacillus buccalis maximus* has been identified by Goadby (47) and by Kligler (72) as an aerobic spore-bearing form belonging in the *Bacillus subtilis* group. Miller's *Leptothrix gigantea*, described, like the others, only on the basis of microscopic study, may have been a true fungus, as Thjøtta, Hartmann and Bøe (137) have suggested.

Leptothrix racemosa is a name given by Vicentini (142) to a peculiar structure, seen in smears from the mouth, consisting of a long thick rod or filament with "conidia" or spore-like bodies clustered around one end. These forms may also have been fungi, or, as Thjøtta and his co-workers believed, artefacts produced by agglomeration of cocci around a leptotrichial or other filament. A somewhat similar form described by Beust (6, 7), likewise on the basis of smears only, consisted of fusiform elements attached to a central stalk so as to give a "test-tube brush" appearance. Beust called this form *Leptothrix falciformis*. Such structures have also been seen by Davis (30) in so-called tonsillar granules and by Goodrich and Moseley (48) and others in pyorrheal pus; and the writer

has seen them occasionally in dark-field preparations of gingival scrapings. An organism with similar morphology was cultivated by Mendel (94) and named *Leptothrix asteroide*. This was a strictly anaerobic gram-negative organism which was said to be pathogenic for animals. Mendel's description and photomicrographs suggest a member of the genus *Bacteroides*. Tunnicliff (140) and Tunnicliff and Jackson (141) isolated from tonsillar granules what seems to have been the same organism, which they named *Vibriothrix tonsillaris*,—a motile gram-negative anaerobe which in pure culture produced rosette and test-tube-brush forms similar to those seen in smears of the original material. This organism was also described as pathogenic for rabbits. Its relationships and significance seem to be entirely unknown.

Leptothrix anaerobius tenuis (80) and *Actinomyces necrophorus* (77) are anaerobic gram-negative filamentous organisms which belong clearly with the genus *Bacteroides*.

Actinomyces muris (138) is a peculiar gram-negative organism of doubtful taxonomic position which, however, can hardly be placed with the true actinomyces without doing further violence to that already abused genus. This organism is the causative agent of one form of rat-bite fever and of other uncommon diseases of man. It was first described by Schottmüller in 1914 (123) under the name *Streptothrix muris ratti*, but has most commonly been called *Streptobacillus moniliformis* (79). The *Haverhillia multiformis* of Parker and Hudson (105) is evidently the same organism. Heilman (57) calls it *Asterococcus muris* and regards it as a bacteria-like phase of a member of the pleuropneumonia group. This group, of which the agent of bovine pleuropneumonia is the typal form, appears to lie parallel with the rickettsiae, but unrelated to them, in a position intermediate between the bacteria as a whole and the viruses. In this taxonomic position the organism would be separated from the actinomyces by the whole range of true bacteria.[2] It appears to inhabit the mouths of rats, mice and other animals, but has not been found in the human mouth, although similar forms have been observed in apparent symbiosis with *Bacteroides funduliformis* (33, 71, 34, 35).

THE BACTERIOLOGY OF ACTINOMYCOSIS

Actinomycosis may be defined as a subacute or chronic, usually progressive disease of cattle and other animals and of man, characterized by the development of indurated granulating swellings particularly in connective tissue, by suppuration usually of limited extent, and by the presence in the pus or lesions of *A. israeli*, demonstrable microscopically, culturally or both.

This is a restricted usage of the term actinomycosis. It follows the recommendations of Wright (153, 154), Colebrook (22) and others, and seems fully justified by available knowledge of the subject. It would exclude somewhat similar diseases in which the causative agent is (a) a saprophytic actinomycete (paractinomycosis, Colebrook 22); (b) *Actinobacillus ligniersi* (actinobacillosis,

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[2] Further data on *Streptobacillus moniliformis* are given in the following references: 68, 69, 70, 71, 33, 32, 119, 147.

Lignières and Spitz, 82, 83); (c) *Staphylococcus aureus* (botryomycosis, Magrou (91); and (d) the clinically distinct cutaneous mycetomas due to *Streptomyces madurae* and other saprophytic forms. In all these diseases club-bearing granules may be found in pus or in the tissues (see Shahan and Davis, 124). Some of them, particularly actinobacillosis,[3] appear to be considerably more common *in cattle* than true actinomycosis, so that diagnosis merely by demonstration of club-bearing granules is unjustified. *In man*, on the other hand, diseases other than true actinomycosis (excepting the cutaneous mycetomas), in which such granules are present, are so rare that demonstration of the granule, commonly accepted as diagnostic by pathologists, is hardly exceptionable.[4] Nevertheless, since clubs, or even the granules themselves, may be lacking in clinically typical instances of actinomycosis in man, which can nevertheless be diagnosed by microscopic demonstration of the branched gram-positive filaments, or better, by recovery of *A. israeli* in pure culture, the presence of club-bearing granules should not be made part of the definition of the disease.

A discussion of the clinical features, histopathology and therapy of actinomycosis is beyond the scope of this review. Colebrook (22), Wright (154) and Cope (25) present authoritative accounts of these aspects of the subject. The use of sulfonamide drugs in therapy has been described by Walker (146), Wilkinson (150), Cutting and Gebhardt (29), and Lyons, Owen and Ayers (87).

Experimental Actinomycosis. *A. israeli*, isolated either from actinomycosis or from the human mouth or throat, has been found capable of reproducing actinomycosis in experimental animals, and has been recovered in pure cultures from the lesions. Such experimental studies have nevertheless been far from decisive, and their results in the aggregate are difficult to evaluate. It has been shown repeatedly that pure cultures of *A. israeli* are at times capable of reproducing in animals lesions in which many of the features of the natural disease are duplicated, including the presence of granules and typical eosinophilic clubs. Yet the majority of such experiments have resulted negatively. Traumatization with a foreign body or by other means has not aggravated the effects of such inoculation, nor has the inclusion of other species of microörganisms in the inoculum. Single inoculations by any of several routes, in many species of animals, with or without traumatic or other manipulation, have resulted at most in the development of localized lesions without the wooden induration or the progressive and fatal character which often characterize the natural disease. Passage from animal to animal has not seemed to increase the virulence of the organism. Repeated inoculation by means apparently involving sensitization has yielded progressive and fatal actinomycosis of a seemingly typical charac-

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[3] Further details on actinobacillosis are given in the following references: 82, 83, 107, 49, 15, 81, 90, 124.

[4] According to Cope (25) only 3 cases of actinobacillosis in man have been recorded. Drake, Sudler and Canuteson (39) have noted that 10 cases of botryomycosis in man have been reported. As noted previously, disease resembling true actinomycosis but due to a saprophytic actinomycete (Colebrook's "paractinomycosis") seems also to be very rare in man.

ter; yet again this result has been obtained without regularity. On the whole the data leave no doubt that *A. israeli* is the causative agent of actinomycosis; that potentially pathogenic actinomyces are widespread in the mouth and throat; and that secondary or accompanying infection is not necessary for the development of the disease. On the other hand the available data do not solve the problem of pathogenesis; they suggest that some condition or combination of conditions as yet unknown determines the occurrence of the disease, but fail to place the mechanism of its development beyond speculation.

Details of experimental actinomycosis may be described under the following heads:

Single inoculation of pure cultures of A. israeli. More or less extensive nodules or tumors, sometimes containing typical granules with clubs, more often containing recoverable actinomyces, have been produced in guinea pigs and rabbits by intraperitoneal inoculation (152, 153), and in rabbits by the intratesticular, subcutaneous or intracutaneous routes (86); in cattle by a single subcutaneous injection (81, 90), or by simultaneous multiple subcutaneous injections (100, 86); in sheep by the subcutaneous route (81); and in swine by inoculation into the testicle or the udder (90). Naeslund (100) also succeeded in infecting a single guinea pig, among several animals injected, by intraperitoneal inoculation. He and others have failed to enhance the virulence of the organism by passage of exudate or recovered cultures. His results and those of Lord and Trevett (86) are noteworthy in that the actinomyces used had been isolated from human tooth-scrapings rather than from actinomycosis. It must be emphasized that none of these workers obtained uniform results. Most of them, for example, failed to produce lesions in guinea pigs and rabbits; and Lignières (81) and Magnusson (90) in particular used a wide range of other animal species with negative or inconclusive results.

Single inoculation with trauma or other manipulation. Traumatization by various means has generally failed to increase the severity of experimental lesions or to yield a more uniform response than simple inoculation. Wright (153) was unconvinced that his positive findings in guinea pigs and rabbits showed clear evidence of multiplication of the organism in the tissues, and attributed the somewhat more extensive lesions produced by Wolff and Israel (152) to the inclusion of agar in the inoculum. Magnusson (90), Naeslund (100), Emmons (41) and Sullivan and Goldsworthy (130) all obtained negative results or atypical lesions by inoculation of cultures and simultaneous implantation of a foreign body such as a horse-hair or a wood-splinter. Grootten (50), who failed to obtain lesions in rabbits after simple inoculation by several routes, succeeded in producing mesenteric nodules by the introduction of agar cultures of *A. israeli* through an incision in the abdominal wall. Lord and Trevett (86) used a similar method in guinea pigs with negative results. Negroni and Bonfiglioli (102) were able to produce indurated abscesses containing recoverable organisms, but apparently not containing granules or clubs, in several rabbits, a guinea pig and a white mouse, all by the subcutaneous route, but only when a suspension of sterile rabbit kidney was added to the inoculum.

Inoculation of unpurified source material containing A. israeli. Magnusson (90) reported that inoculation of animals with pus, derived from actinomycosis in cattle, and containing typical granules, yielded results similar to those obtained with pure cultures. Negroni and Bonfiglioli (102) failed to produce lesions in animals by inoculation of actinomycotic pus. Lord (85) inoculated guinea pigs intraperitoneally with scrapings from carious teeth or with material from tonsillar crypts, both of which had shown actinomyces in sections. In some instances, as would be expected (116) this treatment resulted in generalized peritonitis, probably fuso-spirochetal in character, in which actinomyces probably played no part. In more than half the animals, however, actinomycotic nodules and adhesions developed on the omentum after 2 to 6 weeks, and many of the lesions showed granules with eosin-staining clubs. Somewhat ׀similar results are obtained by Naeslund (100) by intraperitoneal inoculation of guinea pigs with human saliva and tooth scrapings, but in a smaller proportion of animals. Subcutaneous inoculation of cattle with similar mixed material also produced small actinomycotic nodules in some instances. These findings fail to demonstrate that such unpurified inocula are more active in the production of actinomycotic lesions than are pure cultures.

Inoculation of cultures of A. israeli mixed with other bacteria. Pus from actinomycosis often contains contaminating bacteria, particularly when it is taken from a fistula draining to the skin; but there is no reason to believe that such accompanying bacteria need play any part in the natural pathogenesis of the disease. A small aerobic gram-negative organism called *Bacillus actinomycetum comitans* (*Actinobacillus actinomycetem-comitans*, Bergey *et al.* (5)) has been suggested as of special importance in this relation. Klinger (73) originally found this organism in four cases of actinomycosis, and Colebrook (21) found it around granules of *A. israeli* in 24 of 30 cases, but never independently. Pure cultures of the organism killed 2 of 3 rabbits with a quickly developing septicemia after intravenous injection of large doses. Bayne-Jones (4) has also recovered this organism from actinomycosis. Naeslund (100) and Sullivan and Goldsworthy (130) have reported, however, that mixtures of *B. actinomycetum comitans* with *A. israeli* were no more pathogenic for experimental animals than the actinomyces alone. These investigators and also Emmons (41) have also reported failure to produce significant lesions by inoculation of animals with cultures of other bacteria mixed with *A. israeli.*

Repeated inoculation of A. israeli. Progressive and fatal actinomycosis, evidently closely similar in some instances to the natural disease, has been produced in guinea pigs and rabbits by several investigators by repeated inoculation at intervals that might have permitted the development of allergy to the organism. Again, however, the results have been inconsistent and hardly more than suggestive. Mathieson, Harrison, Hammond and Henrici (92) gave as many as six injections of *A. israeli* to five guinea pigs at different intervals up to 46 days, using the intraperitoneal, intratesticular and subcutaneous routes. The animals did not respond to the first injections, but all reacted to later injections in varying degree with inconclusive indications of

actinomycosis. In all these animals, crushed granules from actinomycotic pus were used for some of the inoculations and pure cultures for the remainder. Four additional guinea pigs were inoculated only with pure cultures. One of these was given six injections at irregular intervals over a period of $3\frac{1}{2}$ months, the first intratesticular, the second subcutaneous, and the others intraperitoneal. Another received only two injections, both intraperitoneal, at a 10-day interval. Neither animal showed more than slight local reactions grossly; but both, when killed 7 days after the last injection, showed apparently typical abdominal actinomycosis, with adhesions and multiple nodules containing clubbed granules. Findings in the other two animals, which received 3 and 5 injections respectively, were not characteristic.

These results could not be confirmed by Emmons (41), who gave repeated double inoculations (intraperitoneal and subcutaneous) of *A. israeli* to guinea pigs. As many as 8 such inoculations given at intervals of 4 days to 2 weeks elicited only small abscesses without progressive disease. Negative results were also reported in rabbits by Negroni and Bonfiglioli (102) after two injections of large doses of culture given at 11-day intervals by the intraperitoneal, intravenous or intratesticular routes.

The most striking findings following repeated inoculation of *A. israeli* have been those reported by Slack (126). Positive results were obtained by this worker accidentally following attempts to immunize rabbits with living cultures. The strain of *A. israeli* used had been isolated from pyorrheal pus. Large and increasing doses of a suspension in broth were injected intravenously at 3-week intervals. All of 4 rabbits given 3 or 4 such injections developed indications of chronic progressive disease, fatal in three; while the other was killed a few days after the fourth injection because of extreme emaciation and weakness. At autopsy all four animals were found to have actinomycotic lesions of the viscera, with clubbed granules in three. Similar results were obtained in a single guinea pig, which became emaciated and died after seven intraperitoneal injections of the same culture, given at 3-week intervals. Granules without clubs were found in the viscera of this animal.

Rosebury, Epps and Clark (115) have attempted to confirm the findings of Slack in a larger series of animals. Positive results were obtained with three out of nine strains of *A. israeli*, including two of three isolated from cervico-facial actinomycosis in man, and one of six isolated from gingival scrapings. A total of 24 guinea pigs and 16 rabbits were given from one to eight successive injections at 3-week intervals of massive doses of culture suspended in salt solution. Clear evidence of actinomycosis was obtained in only 5 animals, progressive and fatal in 2 guinea pigs and 1 rabbit, localized and benign in 2 other rabbits. Repeated injection by the intravenous or intraperitoneal routes seemed to be innocuous. Single or repeated subcutaneous injections usually produced only mild local lesions from which the organism could seldom be recovered. The fatal reactions were obtained by intrapleural injection, and by inclusion in the inoculum, given by this route or intravenously, of sterile pulverized salivary calculus. The organism injected was recovered from the lesions

of the fatal cases at autopsy.   In the two guinea pigs with fatal thoracic actino-mycosis granules without clubs were found in sections.   One of the rabbits with localized infection, which showed only small nodules subcutaneously and in the lungs, all of which failed to yield actinomyces on culture, was the only animal in the series in which clubbed granules were found.

These results seem to emphasize the random and uncertain nature of the pathogenesis of actinomycosis.   Repeated inoculation may induce generalized and fatal disease, whereas a single inoculation causes no more than localized lesions with little tendency to progress; yet in both instances the results on the whole are more often negative than positive.   Determining conditions in the pathogenesis of progressive actinomycosis are evidently still unknown.

Attempts to determine whether allergy to *A. israeli* is related to the de-velopment of actinomycosis, by skin tests with living or killed cultures or culture filtrates, have given ambiguous results both in human subjects (92) and in rab-bits (115).   Healthy persons have been found to react irregularly, while pa-tients with frank actinomycosis have failed to react in some instances.   Inocu-lated rabbits reacted more strongly than uninoculated controls, but no clear difference was found between animals which later revealed no pathological changes and one animal that subsequently died with progressive actinomycosis. In man the reactivity of healthy subjects may be related to the occurrence of the organism as an indigenous parasite; and loss of sensitivity may possibly occur with the development of active disease, as is known to happen in tuber-culosis (129).

Epidemiology.   The theory of Bostroem (14) that actinomycosis is an exo-genous infection derived by traumatization with grass, straw or grain which carries the infecting agent has been maintained by repetition in many text books and is still entertained, despite the almost universal failure to adduce evidence in support of it, and the opposite trend of nearly all the available data.   Bostroem found vegetable particles in actinomycotic lesions in man and cattle; he was able, although very rarely, to isolate saprophytic actinomy-cetes from the diseased tissue; but he was unable to demonstrate their patho-genicity.   As noted above, later workers have found that Bostroem's organism is seldom implicated in bovine actinomycosis and is even more rarely found in man.   It has also been assumed that actinomycosis is an occupational disease of farmers and other agricultural workers, transmitted either by contact with infected animals or by direct traumatization with infected grain resulting, for example, from chewing straw.   Davis (31) has reviewed this question and found that such rural groups seem no more subject to the disease than me-chanics, clerks, or urban groups generally.   Among 46 new cases described by Davis, only 15 were farmers and only 3 gave a history of chewing grass or straw.   It has never been clearly shown, moreover, that actinomycosis can be communicated from animals to man, although many instances suggestive of such transmission have been recorded.   Griffith (49) found in England that a high percentage of beef tongues and other tissues from animals slaughtered both locally and in Argentina contained club-bearing granules.   Most of these

were identified as due to actinobacillosis, but in more than 5 per cent of instances they appeared to be true actinomycetes. Yet no instance of actinomycosis in man has ever been traced clearly to the ingestion of such contaminated food. Transmission from man to man seems to have been suggested in only two instances (2, 88) but without convincing evidence in either.

The causative agent of true actinomycosis, *A. israeli*, has never been found apart from a parasitic or pathogenic habitat; and its inability to grow at low temperatures, its lack of spores, and its requirement for reduced oxygen tension, all seem to make it incapable of existence under saprophytic conditions. It has been noted that potentially pathogenic *A. israeli* can frequently be found in the human mouth and throat in such local disorders as chronic tonsillitis, dental caries, gingivitis and pyorrhea, in the etiology of which these organisms appear to play no part. It is possible that they occur in small numbers in the fully healthy mouth; and that, in common with a large section of the mouth flora, they proliferate under a variety of different local conditions which contribute to or constitute the picture of poor oral hygiene. It seems justifiable, at all events, to include them with such microörganisms as the lactobacilli and the oral spirochetes (neither of which can be demonstrated constantly in the mouth) as members of the indigenous flora, and thus to assume that the oral and pharyngeal mucous membranes are their natural habitat. Since actinomyces isolated from such sources evidently belong in the same group as those obtained from clinical disease, and since strains from both sources have been found capable of producing experimental actinomycosis, the endogenous character of the natural disease seems inescapable. This view was first suggested by Wolff and Israel (152) and amplified by Wright (153). It has since come to be accepted virtually without dissent by all students of the parasitic actinomycetes. Naeslund (100) and others have suggested, moreover, that if trauma from straw, splinters and other vegetable matter plays a part in the pathogenesis of actinomycosis, it may be by facilitating the introduction of *A. israeli* into the tissues from a mucous membrane, or by aiding the growth of this organism in the tissues, rather than by causing exogenous infection with a saprophytic actinomycete.

Just how infection of adjacent or remote tissues develops through the agency of natural surface parasites nevertheless remains to be determined. It may be noted here that the comparative rarity of actinomycosis in man seems in line with the experimental data previously given in suggesting that the determining factor in the disease is something in addition to and less common than local trauma or even repeated autoinoculation, although both may well be contributory incidents in its pathogenesis. It may be significant that a history of tooth extraction or other injury to mouth or throat has frequently been obtained in cervico-facial actinomycosis (see Cope, 25; Davis, 31); that three instances of hand infection with actinomycosis following wounds inflicted by the human teeth have been recorded (89, 24, 112A); that pulmonary actinomycosis has been associated with aspiration of an extracted carious tooth or tooth fragment (61A, 148); and that an actinomycete has been recovered from the

blood immediately after extraction of a tooth (104). Finally, the apparent rôle of *A. israeli* in the deposition of salivary calculus, as detailed below, suggests that these deposits may play a part in the pathogenesis of actinomycosis. The experimental data of Rosebury, Epps and Clark (115) tend to support this view, but it has not been clearly demonstrated. This general subject invites further investigation, and currently improved methods for the cultivation and maintenance of *A. israeli*, as described above, may facilitate its solution.

Diagnosis. Diagnosis of actinomycosis in man rests on a combination of clinical signs and on microscopic demonstration, or preferably isolation, of the causative agent. Attempts to apply other specific diagnostic tests, such as the demonstration of agglutinins to *A. israeli* in the patient's serum (21) or of skin sensitivity to the organism or its products (92) have not been successful. Neuber (103) has recommended both a complement-fixation reaction and a skin test, but the conditions given for performing these tests make them appear impracticable. It is apparent, therefore, that no means of specific diagnosis is available in the absence of surface lesions, or where exudate cannot be obtained for bacteriological study.

Direct microscopic demonstration of *A. israeli* depends on the presence of gram-positive branched rods or filaments. Eosinophilic clubs are a confirmatory but not an indispensable adjunct. Isolation of *A. israeli* in pure culture, however, is the most convincing diagnostic procedure.

Where exudate containing typical granules can be obtained with reasonable expectation that contaminating bacteria are absent, inoculation of glucose-agar shake cultures may suffice for isolation. The granule is transferred to a tube of melted agar that has been cooled to about 45°C, broken against the wall of the tube, and distributed through the medium. Several additional tubes of melted glucose agar should then be inoculated serially. These cultures may be incubated at 37°C in air. After 3 to 6 days, successful uncontaminated cultures show the characteristic whitish spherical or mulberry-like colonies growing in the depths of the agar, often with a dense zone of colonies about a centimeter below the free surface. The diagnosis is confirmed by demonstration in such a colony, removed with a capillary pipette, of a branched mycelium or of branched twig-like gram-positive rods and short filaments.

The method used by Rosebury, Epps and Clark (115), although not so simple as the preceding, is recommended as preferable, and is almost indispensable if the source material is contaminated. For this purpose Bacto brain-heart medium containing 2 per cent of agar is used in streaked plates. The granule, or a loopful of exudate or other source material, without washing or other manipulation, is streaked serially on four plates of this medium with a bent glass rod. A loopful of sterile broth may be used on each plate to help moisten and distribute the inoculum. The plates are incubated for 4 to 6 days at 37°C in an anaerobic jar with hydrogen, catalyzed by heated platinum or palladium, and containing about 5 per cent of carbon dioxide. Typical colonies may then

be found and identified as described previously.[5] A typical colony should be fished to a glucose-agar shake tube and the subculture used for confirmation as described above.

Prevention. Since we are ignorant of the mechanism whereby *A. israeli* proceeds from its habitat on the mucous membranes to set up progressive lesions in the deeper tissues, no specific recommendations for prevention can be given. In general the data seem to constitute one reason among others for the avoidance of excessive trauma in surgery of the mouth and pharynx. In view of the indications that the organism is found particularly in the presence of local inflammatory processes like pyorrhea or gingivitis, the prevention or control of these disorders may be expected to help prevent actinomycosis, among other possible systemic sequels of defective oral hygiene.

SALIVARY CALCULUS

There is convincing evidence that microörganisms play an important part in the deposition of salivary calculus or tartar,—the calcified masses that form on the surfaces of teeth or other fixed structures (such as prosthetic appliances) in the mouth. Much of the evidence points to *Actinomyces israeli* as the agent in the formation of these concretions; but the data leave many questions unanswered, particularly with reference to the mechanism whereby the deposits are formed.[6]

The several clinical varieties of tartar—supragingival and subgingival calculus and salivary duct stones—all appear to be similar in chemical composition (74) and in microscopic structure (98, 99), as well as in their content of microorganisms (99, 8). They all seem to originate from saliva. Physical differences between concretions from the different sites, in hardness, texture and color, are probably dependent on the location in which the mass is deposited (75). *Supragingival calculus*, the variety that forms in largest amount and is most readily obtained for study, is deposited on exposed surfaces of the teeth, preferentially on those adjacent to the orifices of the salivary ducts; i.e., on the lingual surfaces of the lower anterior teeth and on the buccal surfaces of the upper first and second molars. It is also found frequently on malposed or irregular tooth surfaces elsewhere, in areas that are difficult to reach or are habitually not reached by the toothbrush (59), and on the surfaces of teeth that are not used in chewing because of pain or because opposing teeth are lacking. When freshly deposited these concretions are cream-colored or yellowish and soft, so that they may easily be brushed away (13); but they become hard and firmly adherent within a few days, and later they become stained by food or tobacco. *Subgingival calculus*, sometimes miscalled "serumal" calculus, is

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[5] This method has been found successful for isolation of *A. israeli* both from pus from actinomycosis and from grossly contaminated sources such as gingival scrapings. It should be noted that in view of the sensitivity of the method and of the frequent occurrence of the organism in the mouth and throat, the recovery of *A. israeli* from sputum or other material contaminated from these areas is not necessarily diagnostic of actinomycosis.

[6] For general reviews of salivary calculus see Rosebury and Karshan (117) and Tenenbaum and Karshan (136).

deposited on the tooth surface under the free margin of the gum within the confines of the gingival crevice or in a pyorrheal pocket. It is generally harder than the supragingival form, perhaps because it is formed more slowly, and is usually deeply stained, probably as a result of small hemorrhages around it. This form of tartar is found characteristically in pyorrheal pockets, but it is not known whether it is a cause or an effect of the pocket. Supragingival calculus may cause irritation of the underlying epithelium, but because of its position on the outer gingival epithelium it probably has no bearing on pocket formation. That both varieties may be irritating, however, and may aggravate gingival and periodontal lesions if they do not cause them, is indicated by the beneficial results that generally follow their removal.

The inorganic composition of tartar and duct stones has been studied by several investigators (131, 74, 108, 64, 45) with a good agreement in the data which, especially in view of differences in analytical methods, suggests that the concretions originate by a uniform process. Mineral matter constitutes about 70 to 80 per cent of the mass, and consists mainly of calcium and phosphorus, with magnesium and other elements in smaller amounts. The proportions of calcium, phosphorus and magnesium in tartar are similar to those in dentin, and appear to be present either as tricalcium phosphate or as a hydroxyapatite or other apatite-like salt (64, 106, 93). Chemical study of the organic portion of subgingival tartar (45) has indicated about 8.3 per cent of protein, consisting of keratin, mucin and nucleoprotein; and 2.7 per cent of fatty material. The keratin and mucin appear to be derived by inclusion in the mass of desquamated epithelial cells and of saliva, respectively.

Microörganisms in Calculus. All three clinical varieties of calculus, when studied in carefully decalcified sections stained by Gram's method, have been found to contain a characteristic stroma of filamentous microörganisms, in parts of which true branching can be seen (98, 99, 128, 51, 52, 53, 54, 133). According to Naeslund, this stroma can be demonstrated in all specimens of tartar if careful histological methods are used. Branching filaments are seen partieularly in the deeper or older portions of the mass, while continuous with these in the more peripheral or newer portion the stroma may consist of unbranched filaments in palisade or radial arrangement. In some instances, however, only branching filaments can be seen, and these pervade the entire mass. Naeslund reported that typical actinomycotic clubs may be demonstrable occasionally in tartar, especially in the subgingival variety.

Bacteriological studies of tartar always yield a variety of indigenous oral microörganisms, as would be expected. It seems significant, however, that *Actinomyces israeli* has been isolated both from the surface and from the depths of tartar more often than other filamentous microörganisms, and that it appears to be the only organism among those commonly recovered whose morphology would account for the characteristic branched stroma seen in sections. Organisms whose properties as they were described indicate their identity with *A. israeli* have been isolated from tartar by Bulleid (18, 19), Naeslund (99), Bibby and Knighton (10, 11); Grythe (52, 53, 54), and Rosebury, Epps and Clark

(115). Naeslund, whose findings seem particularly noteworthy in view of his earlier (97) careful studies of actinomycetes, was able to isolate anaerobic actinomyces repeatedly from both supragingival and subgingival concretions, more often from the peripheral calcified portion than from the deeper portion. He noted that such organisms could be recovered much more commonly than the unbranched filamentous forms. He and others have called such unbranched forms *Leptothrix* or *Leptotrichia*. As noted above, organisms from tartar which have been thus named cannot be clearly identified, and they appear to play no important part in the formation of the deposits. An exception is the organism isolated by Bulleid (18, 19). Although he called it *Leptothrix buccalis*, Bulleid's description of a gram-positive form showing true branching, preferring anaerobic conditions, and growing as small adherent raised rough colonies, suggests that he was dealing with *A. israeli*.

That microörganisms play an essential part in the deposition of tartar is indicated by the findings of several investigators, who were able to produce deposits similar to natural tartar *in vitro*. Such artificial deposits have appeared in stagnating saliva (108, 99, 55); in artificial mixtures of salts and protein made to resemble saliva and left exposed to air and contamination (108); and in culture media containing calcium salts and inoculated with pure cultures of actinomycetes or other organisms (18, 19, 99, 8). In the latter experiments artificial tartar deposition did not occur in sterile media, and was inhibited by the addition of disinfectants or when killed instead of viable organisms were inoculated. As noted above, the organism studied by Bulleid appears to have been *A. israeli*. Naeslund also produced concretions with pure cultures of actinomyces, and apparently succeeded likewise with his unidentified "leptothrix." Bibby reported best results with unidentified filamentous organisms and with *Proteus sp.* and *Bacillus subtilis*. The data of Bulleid and Naeslund suggest the capacity of *A. israeli* to form concretions, but the evidence as a whole leaves little doubt that other organisms may be equally capable of forming them. It seems noteworthy that *A. israeli* is not conspicuous for its calcifying action in most other areas. The "sulfur granules" of actinomycosis are only rarely calcified (81). Cornell (26) has described a case of actinomycosis of the internal female genitalia in which the granules, found in sections, seemed to have been composed largely of unidentified crystals. Elliot (40) noted the occurrence of calcified sulfur granules in actinomycosis of the lachrymal canaliculi. Of special interest in this connection is the view of Söderlund (128) that salivary duct stones are formed as a result of a localized actinomycotic infection of the ducts. Precipitation of calcium salts by *A. israeli*, although by no means a constant phenomenon, may be conditioned by the environment in which the organism grows.

Mechanism of Tartar Deposition. The manner in which calculus is deposited, whether by the action of *A. israeli* or otherwise, has been the subject of much speculation and experiment but has thus far remained *obscure.* The older theories of tartar formation have been discussed by Rosebury and Karshan (117), and may be reviewed here briefly. The belief that loss of carbon dioxide

from saliva is the essential cause of tartar formation (96, 67) is invalidated by the findings (a) that the change in CO_2 content of saliva on exposure to air is insignificant despite loss of the gas (20), and (b) that limitation of the escape of CO_2 from stagnating saliva *in vitro* has not affected the formation of artificial deposits (55, 99). The theory of Prinz (108) which suggests that precipitation depends on loss of protective colloid as a result of surface concentration of colloids in stagnating saliva fails to take account of bacterial action, as disclosed by subsequent studies, and rests on the doubtful assumption that salivary stagnation is essential to the process. It seems hardly likely that the oral regions nearest the salivary duct orifices, in which tartar forms by preference, afford better conditions for stagnation than other parts of the mouth. Broderick's view (16) that tartar deposition depends upon a generalized alkalosis which is reflected in increased salivary alkalinity has received little support from the data of Tenenbaum and Karshan (134, 135, 136), who found the difference in pH between calculus-free and calculus-forming groups of persons to be small and of uncertain significance in stimulated saliva, while the average pH values in unstimulated saliva for the two groups were nearly identical. Changes in the pH of the medium, moreover, have not been found important in the deposition of artificial concretions (55, 8).

Naeslund (99) has suggested that calculus is formed through the activity of actinomyces and "leptothrix," which induce the precipitation of salivary salts and also act as a matrix both to retain the deposit and to attach it to the tooth surface. Since the organisms remain alive at the periphery of the calcified mass, the process would be progressive rather than self-limiting. This part of Naeslund's theory—except for its inclusion of the ambiguous "leptothrix"— is in good agreement with the facts presented above. The known ability of *A. israeli* to form a dense branched mycelium which tends to attach itself firmly to the surface on which it grows may, indeed, make this organism uniquely capable of acting as a matrix for the deposition of tartar.

Naeslund goes further, however, to suggest that the mechanism whereby these microörganisms precipitate salivary salts depends, first, on the loss of salivary colloids, and hence of their "protective effect" on the salts in solution, as a result of bacterial proteolysis; and secondly, on a consequent or correlated increase in the pH of the medium. As noted previously, Naeslund (97) reported that some strains of *A. israeli* were proteolytic and could induce an alkaline reaction; but other workers have uniformly failed to confirm this finding. Naeslund himself (99) found that the proteolytic action of his "leptothrix" was "weak, in some cases uncertain," and stated that "on account of the scanty growth no definite change in reaction could be detected" with this organism. There is no doubt that the mechanism postulated by Naeslund is theoretically sound, in that the changes he suggested would result in the precipitation of salivary calcium salts; but the data do not make it clear that the microorganisms concerned are capable of inducing them.

An alternative explanation, involving the action of phosphatases, may perhaps be substituted for the doubtful part of Naeslund's theory. The evidence

on this point, however, is incomplete and hardly more than suggestive. The action of these enzymes, which liberate phosphate from organic combination and are known to take part in the deposition of the calcified tissues, was first suggested as important in tartar formation by Adamson (1) and Smith (127). These workers believed that phosphatase derived from the gingival tissue rather than from microörganisms is the active agent in the process. Zander (155), however, has shown by a histochemical method that phosphatase is not present in gingival epithelium, although it is found in the connective tissue, and particularly in capillary endothelium. Phosphatase would thus be liberated from the gums only under pathological conditions. On the other hand it is known that microörganisms may produce phosphatase. Glock, Murray and Pincus (46) found it produced by several oral species, including strains of actinomycetes which, however, appear to have been saprophytes rather than *A. israeli*. Smith (127) had shown that phosphatase activity in saliva was concentrated in the sediment after centrifugation; and, as Zander (155) has pointed out, this finding may indicate that the greater part of the activity in saliva is derived from the oral microörganisms. The phosphatase activity of many bacteria may explain their apparent ability to produce concretions *in vitro*, as noted above. Yet it has not been shown that *A. israeli* produces phosphatase, and no direct evidence has been provided to link these enzymes to tartar formation. Saltzmann (121) and Tascher and Wagreich (132) have both found that the degree of salivary phosphatase activity is not related to the presence or characteristic absence of tartar on the teeth. The enzyme that may be associated with tartar formation, however, would be expected to constitute no more than a small fraction of the total phosphatase content of saliva at large; hence the negative import of these results may not be significant.

Smith (127) and Glock, Murray and Pincus (46) found maximal phosphatase activity in saliva in the pH range 5.0 to 6.0; while Tascher and Wagreich (132) have reported phosphatase activity both at pH 4 and at pH 7.4 in a sample of supragingival calculus. The latter workers also observed that human parotid saliva collected directly from the orifice of Stenson's duct showed phosphatase activity. Although their experiment does not necessarily exclude microorganisms as the source of the enzyme, it seems improbable that salivary phosphatase is derived entirely from the microörganisms of the mouth.

If *Actinomyces israeli* is the active agent in the deposition of calculus, its capacity to form tartar would seem to depend chiefly on its morphology and characteristic growth which, as Naeslund suggested, would attach the growing mass to the tooth surface and serve as a stroma or matrix for continued formation of the deposit. Being unique in this respect, it might not need to be unique in possessing a capacity to precipitate calcium salts, whatever the mechanism of such precipitation may be, in order to serve as the only agent in which all the necessary properties for calculus deposition are combined. A clear demonstration of the capacity of *A. israeli* to form tartar, and of the means it employs to do so, is nevertheless needed to make this suggestion something more than the best guess afforded by the available information.

Predisposing Factors in Tartar Formation. Salivary calculus is seldom found in the mouths of children, but its occurrence seems to increase with advancing age, and it is very prevalent among adults. It has been found on the teeth of Egyptian mummies, Incas and other ancient and aboriginal peoples (66), as well as on those of modern primitives such as Eskimos (118) and other isolated groups such as the natives of Tristan da Cunha (122, 44). Such groups are known to be comparatively free from dental caries; and it is a matter of common observation that mouths with particularly heavy tartar deposits may be found free from decay. Rosebury and Waugh (118) reported that among a group of Alaskan Eskimos those with active caries generally had less tartar and those free from caries generally had more tartar than would have been expected on the basis of their average age alone. It is of interest, moreover, that groups of persons who tend habitually to form tartar in large amounts have been found by Tenenbaum and Karshan (134, 135, 136) to have significantly higher levels of total calcium and inorganic phosphorus, in both stimulated and unstimulated saliva, than groups lacking this tendency. Similar findings have been reported for Alaskan Eskimos (63, 65) and for Greenland Eskimos (62). The differences were evidently not due to the presence of large calcified masses in the mouths of the group with tartar, since similar values were obtained before and after removal of the deposits (135). The protein content of saliva was not found significantly different in the two groups. In stimulated saliva the average pH was slightly lower for the calculus-free group, the difference between the two groups being of uncertain significance; while the average values for pH of unstimulated saliva from the two groups were nearly identical.

Willsmore (151) has reported that the occurrence of calculus is related to oral hygiene, indicated in terms of the frequency of tooth brushing, and also to variation in tendency of saliva to deposit cellular débris on standing. Among 36 persons who brushed their teeth at least twice a day, and whose saliva did not tend to deposit débris, none had calculus. At the other extreme, among 51 persons who never used the toothbrush, and whose saliva tended markedly to deposit débris, all but one had calculus, and 45 had abundant deposits. Data for groups with intermediate degrees of these two conditions were generally consistent with this pattern.

Since tartar is soft and easily removed when it is first formed, the development of hard deposits can presumably be prevented by regular and efficient brushing of the teeth. Otherwise, however, it appears that a tendency to form calculus may depend on individual variation in the composition of saliva. There are no data on the incidence of *A. israeli* in the mouth correlated with such tendency. It has been noted that persons with tartar generally have a higher salivary concentration of the principal inorganic constituents of the deposit. This finding may imply that such higher concentrations are required in order that the mechanism of precipitation may become operative.

Tartar in itself (excluding duct stones), in view of its widespread occurrence, can hardly be looked upon as pathological; but on clinical grounds it seems

clear that it may lead to or aggravate pathological changes in the adjacent soft tissues. The occurrence of viable *Actinomyces israeli* in tartar has led to the suggestion (23) that detached masses of this material may be instrumental in the pathogenesis of actinomycosis, whether by aspiration into the respiratory tract or by other means; and as noted previously suggestive evidence in support of this view has been provided from experimental studies (115). It is evident, however, that only further research can clarify the many questions in this field that remain unanswered.

SUMMARY

The name *Actinomyces israeli* is applied in this review in accordance with priority and usage to those gram-positive, branched microörganisms which are generally cultivable under partial anaerobiosis, which are parasitic on certain mucous membranes, and which are responsible for true actinomycosis in man and animals. The morphology of this organism is described as it appears in sections of and in exudates from the lesions of actinomycosis, and also in preparations from the human mouth and throat, and in cultures. Methods are given for its cultivation and maintenance; and its cultural characteristics are described under various conditions of growth. It is noted that *A. israeli* is a heterogeneous species, but that attempts to subdivide it have not as yet given satisfactory results. Interrelationships are considered of *A. israeli* with bacteria and fungi in general, with the anaerobic lactobacilli, and with the saprophytic actinomycetes.

Leptotrichia buccalis, an unbranched gram-positive parasite of the human mouth, is described, and data are reviewed which suggest that it has no pathogenic significance. Other microörganisms, described as leptotrichia, actinomycetes, or under other names, are considered in an effort to place them taxonomically. These include *Leptotrichia* (*Cladothrix*) *placoides*, *Leptothrix racemosa* and *L. falciformis*, and *Actinomyces muris*.

Actinomycosis is treated with special reference to the experimental production of the disease in animals. Data are given which indicate that single inoculations of pure cultures of *A. israeli* have yielded non-progressive lesions irregularly; trauma or other manipulation with such single inoculations has not seemed to aggravate this effect; nor has the use for inoculation of pus from naturally occurring actinomycosis, or of other unpurified material containing the causative organism. Supplementing pure cultures of *A. israeli* with *Bacillus actinomycetum comitans* or other bacteria has likewise failed to enhance the infectivity of the inoculum. On the other hand, progressive and fatal actinomycosis has been produced experimentally, in rabbits and guinea pigs by repeated injection of *A. israeli*. Here again, however, the results have been irregular and suggest that essential factors in the pathogenesis of actinomycosis are still unknown. It has not been clearly demonstrated that allergy to *A. israeli* is such a factor. The common occurrence of potentially pathogenic *A. israeli* on the oral and pharyngeal mucous membranes in the absence of actinomycosis, the apparently strictly parasitic habit of the organism, and most of the avail-

able epidemiological data all point to an endogenous origin of the natural disease.

Methods for the bacteriological diagnosis of actinomycosis are given, and the problem of prevention of the disease is considered briefly.

The data reviewed on the nature, composition and manner of formation of salivary calculus or tartar indicate that these concretions are precipitated from saliva through the action of oral microörganisms. Suggestive but incomplete evidence points to *Actinomyces israeli* as the causative agent. The mechanism of tartar deposition, although the subject of much speculation, has not been clarified. The hypothesis that seems most promising involves the action of phosphatase of microbial origin. It is suggested that the capacity of *A. israeli* to grow as a branched mycelium attached to tooth surfaces, by providing a stroma for tartar deposition, may make this organism peculiarly capable of forming calculus. The saliva of persons who tend to form tartar has been found to contain higher average concentrations of calcium and inorganic phosphorus than the saliva of non-formers, and it is possible that such high levels may be required for their precipitation.

BIBLIOGRAPHY

(1) ADAMSON, K. T. 1929 The rôle of enzyme action in the formation of dental calculi. Australian J. Exptl. Biol. Med. Sci., 6, 215–227.

(2) BARACZ, R. V. 1889 Übertragbarkeit der Aktinomykose vom Menschen auf den Menschen. Wien. med. Presse, 30, 6–11.

(3) BARTELS, H. A. 1943 A filamentous microörganism isolated from stained teeth. J. Dental Research, 22, 97–102.

(4) BAYNE-JONES, S. 1925 Club-formation by *Actinomyces hominis* in glucose broth, with a note on *B. actinomycetum-comitans*. J. Bact., 10, 569–575.

(5) BERGEY, D. H., *et al.* 1939 Bergey's Manual of Determinative Bacteriology. Williams & Wilkens Co., Baltimore, 5th ed.

(6) BEUST, T. 1908 A contribution of the morphology of the microörganisms of the mouth. Dental Cosmos, 50, 594–595.

(7) BEUST, T. B. 1937 Morphology of the fusiform bacillus. J. Dental Research, 16, 379–386.

(8) BIBBY, B. G. 1935 The formation of salivary calculus. Dental Cosmos, 77, 668–676.

(9) BIBBY, B. G., AND BERRY, G. P. 1939 A cultural study of filamentous bacteria obtained from the human mouth. J. Bact., 38, 263–274.

(10) BIBBY, B. G., AND KNIGHTON, H. T. 1935 Mouth organisms resembling actinomyces. J. Dental Research, 15, 171.

(11) BIBBY, B. G., AND KNIGHTON, H. T. 1941 The actinomyces of the human mouth. J. Infectious Diseases, 69, 148–154.

(12) BIGGART, J. H. 1934 Actinomycosis graminis. Bull. Johns Hopkins Hospital 54, 165–173.

(13) BLACK, G. V. 1911 Beginnings of pyorrhoea alveolaris treatment for prevention. Dental Items of Interest, 33, 420–455.

(14) BOSTROEM, E. 1891 Untersuchungen über die Aktinomykose des Menschen. Beitr. path. Anat., 9, 1–240.

(15) BOSWORTH, T. J. 1923 The causal organism of bovine actinomycosis. J. Comp. Path. Therap., 36, 1–22.

(16) BRODERICK, F. W. 1936 Principles of Dental Medicine, the Medical Aspects of Dental Disease. C. V. Mosby, St. Louis, 2nd ed.

(17) BUEHLMANN, F. 1840 Über eine eigenthümliche, auf den Zähnen des Menschen vorkommende Substanz. Arch. Anat., Physiol. wiss. Med., 442–445.

(18) BULLEID, A. 1924 An experimental study of *Leptothrix buccalis*. Guy's Hosp. Rept., 74, 444–458.

(19) BULLEID, A. 1925 An experimental study of *Leptothrix buccalis*. Brit. Dental J., 46, 289–300.

(20) CLARK, C. W., AND CARTER, K. L. 1927 Factors involved in the reaction changes of human saliva. J. Biol. Chem., 73, 391–404.

(21) COLEBROOK, L. 1920 The mycelial and other micro-organisms associated with human actinomycosis. Brit. J. Exptl. Path., 1, 197–212.

(22) COLEBROOK, L. 1921 A report upon 25 cases of actinomycosis, with especial reference to vaccine therapy. Lancet 200, 893–899.

(23) COLEBROOK. L. 1931 The anaerobic actinomyces. A system of Bacteriology in Relation to Medicine. Medical Research Council, London, 8, 78–83.

(24) COPE, V. Z. 1915 A clinical study of actinomycosis, with illustrative cases. Brit. J. Surg., 3, 55–81.

(25) COPE, Z. 1938 Actinomycosis. Oxford University Press, London.

(26) CORNELL, V. H. 1934 Actinomycosis of tubes and ovaries. Am. J. Path., 10, 519–530.

(27) CRECELIUS, H. G. AND RETTGER, L. F. 1943 The intestinal flora of the guinea pig. J. Bact., 46, 1–13.

(28) CROWLEY, M. C. 1941 The isolation of an actinomycetes-like organism from root canals. J. Dental Research, 20, 189–194.

(29) CUTTING, W. C., AND GEBHARDT, L. P. 1941 Inhibitory effects of sulfonamides on cultures of *Actinomyces hominis*. Science, 94, 568–569.

(30) DAVIS, D. J. 1914 The actinomyces-like granules in tonsils. J. Infectious diseases, 14, 144–158.

(31) DAVIS, M. I. J. 1941 Analysis of forty-six cases of actinomycosis with special reference to its etiology. Am. J. Surg., 52, 447–454.

(32) DAWSON, M. H., AND HOBBY, G. L. 1939 Rat-bite fever. Trans. Assoc. Am. Physicians, 54, 329–332.

(33) DIENES, L. 1939 L type variant forms in cultures of various bacteria. Proc. Soc. Exptl. Biol. Med., 42, 636–640.

(34) DIENES, L. 1942 The significance of the large bodies and the development of L type of colonies in bacterial cultures. J. Bact., 44, 37–73.

(35) DIENES, L., AND SMITH, W. E. 1942 Relationship of pleuropneumonia-like (L) organisms to infections of human genital tract. Proc. Soc. Exptl. Biol. Med., 50, 99–101.

(36) DOBELL, C. 1932 Antony van Leeuwenhoek and his "little animals". Harcourt, Brace & Co., New York.

(37) VON DOBRZYNIECKI, A. R. 1937 Zwei chromogene Mikroorganismen der Mundhöhle. Zentr. Bakt. Parasitenk. Abt. I, 21, 833–835.

(38) VON DOBRZYNIECKI, A. R. 1898 Beitrage zu Bakteriologie der Zahncaries. Zentr. Bakt., Abt. I, 23, 976–979.

(39) DRAKE, C. H., SUDLER, M. T., AND CANUTESON, R. I. 1943 A case of staphylococci actinophytosis (bothyomycosis) in man. The tenth reported human case. J. Am. Med. Assoc., 123, 339–341.

(40) ELLIOT, A. J. 1941 Streptothricosis of the lacrimal canaliculi—Report of 9 cases. Am. J. Ophthalmol., 24, 682.

(41) EMMONS, C. W. 1938 The isolation of *Actinomyces bovis* from tonsillar granules. U. S. Pub. Health Repts., 53, 1967–1975.

(42) ERIKSON, D. 1935 The pathogenic aerobic organisms of the actinomyces group. Med. Research Council (Brit.) Special Rept. Series No. 203.

(43) ERIKSON, D. 1940 Pathogenic anaerobic organisms of the actinomyces group. Med. Research Council (Brit.), Special Rept. Series No. 240.

(44) FERGUSON, R. A. 1934 A dental survey of the school children of American Samoa.
J. Am. Dental Assoc., 21, 534–549.

(45) GLOCK, G. E., AND MURRAY, M. M. 1938 Chemical investigation of salivary calcu-
lus. J. Dental Research, 17, 257–264.

(46) GLOCK, G. E., MURRAY, M. M., AND PINCUS, P. 1938 The origin and significance of
salivary phosphatase. Biochem. J., 32, 2096–2104.

(47) GOADBY, K. W. 1903 The Mycology of the Mouth. Longmans, Green & Co.,
London.

(48) GOODRICH, H. P., AND MOSELEY, M. 1916 On certain parasites of the mouth in
cases of pyorrhea (preliminary communication. J. Roy. Microscop. Soc., 513–
527.

(49) GRIFFITH, F. 1916 On the pathology of bovine actinomycosis. J. Hyg., 16, 195–
207.

(50) GROOTEN, O. 1934 Caractéres généraux et pouvoir pathogène expérimental de
l'*Actinomyces israëli*. Ann. inst. Pasteur, 53, 311–323.

(51) GRYTHE, O. 1933 Spiller visse sopparter en rolle for tannstersdannelse. Norske
Tannlaegeforenings Tidende, 43, 1–6.

(52) GRYTHE, O. 1938 Leptotrix. Experimentelle Untersuchungen an Leptotrix die
aus klinisch gesunden Zahnfleisch gezüchtet wurden. Fabritius & Sønner, Oslo.

(53) GRYTHE, O. 1938 The rôle of Actinomyces and Leptothrix as an etiological factor
in the formation of tartar and salivary calculi. Norske Tannlaegiforenings
Tidende, 48, 433–447.

(54) GRYTHE, O. 1938 Leptothrix. Experimentelle Untersuchungen an Leptothrix die
aus Klinisch gesunden Zahnflesich gezüchtet wurden. Norske Tannlaegeforenings
Tidende, 48, Suppl. No. 5.

(55) HALL, I. C., AND WESTBAY, C. 1925 Bacterial factors in pyorrhea alveolaris. (II)
Further studies on reaction changes in saliva, due to microörganisms, and their
possible relation to the formation of dental calculus. Dental Cosmos, 67, 115–
124.

(56) HARZ, IN BOLLINGER, O. 1877 Ueber eine neue Pilzkrankheit beim Rinde. Zentr.
med. Wiss., 15, 481–485.

(57) HEILMAN, F. R. 1941 A study of *Asterococcus muris (Streptobacillus moniliformis)*
1. morphologic aspects and nomenclature. J. Infectious Diseases, 69, 32–44.

(58) HENRICI, A. T. 1930 Molds, Yeasts and Actinomycetes. John Wiley & Sons, New
York.

(59) HIRSCHFELD, I. 1939 The Toothbrush: Its Use and Abuse. Dental Items of In-
terest Publishing Co., Brooklyn, N. Y.

(60) HOLM, P. 1930 Comparative studies on some pathogenic anaërobic actinomyces.
Acta Path. Microbiol. Scand., Suppl. 3, 151–156.

(61) ISRAEL, J. 1878 Neue Beobachtungen auf dem Gebiete der Mykosen des Menschen.
Virchow's Arch., 74, 15–53.

(61A) ISRAEL, J. 1886 Ein Beitrag zur Pathogenese der Lungenaktinomykose. Ver-
handl. deut. Ges. Chir. 15, part 2, 36–40.

(62) KARSHAN, M., PEDERSEN, P. O., SIEGEL, E. H., AND TENENBAUM, B. 1940 Bio-
chemical studies of the saliva of Greenlanders correlated with dental caries and the
occurrence of salivary calculus. J. Dental Research, 19, 303–304.

(63) KARSHAN, M., ROSEBURY, T., AND WAUGH, L. M. 1939 Dental caries among Eski-
mos of the Kuskokwim area of Alaska. II. Biochemical characteristics of stimu-
lated saliva correlated with dental caries and occurrence of salivary calculus. Am.
J. Diseases Children, 57, 1026–1034.

(64) KARSHAN, M., AND SCHROFF, J. 1928 Composition of some salivary calculi. J.
Dental Research, 8, 454–455.

(65) KARSHAN, M., SIEGEL, E. H., AND WAUGH, L. M. 1940 Biochemical studies of the
saliva of Eskimos correlated with dental caries and the occurrence of salivary calcu-
lus. Am. J. Diseases Children, 59, 39–44.

(66) KELSEY, C. J. 1914 Dental calculus. Brit. Dental J., 35, 810–817.

(67) KIRK, E. C. 1911 American Textbook of Operative Dentistry. Lea & Febiger, Philadelphia, 4th ed.

(68) KLIENEBERGER, E. 1935 The natural occurrence of pleuropneumonia-like organisms in apparent symbiosis with *Streptobacillus moniliformis* and other bacteria. J. Path. Bact., 40, 93–105.

(69) KLIENEBERGER, E. 1936 Further studies on *Streptobacillus moniliformis* and its symbiont. J. Path. Bact., 42, 587–598.

(70) KLIENEBERGER, E. 1938 Pleuropneumonia-like organisms of diverse provenance: some results of an enquiry into methods of differentiation. J. Hyg., 38, 458–476.

(71) KLIENEBERGER, E. 1940 The pleuropneumonia-like organisms: further comparative studies and a descriptive account of recently discovered types. J. Hyg., 40, 204–222.

(72) KLIGLER, I. J. 1915 A biochemical study and differentiation of oral bacteria with special reference to dental caries. J. Allied Dental Soc., 10, 141–166; 282–330; 445–458.

(73) KLINGER, R. 1912 Untersuchungen über menschliche Aktinomykose. Zentr. Bakt. Parasitenk., Abt. I, Orig., 62, 191–200.

(74) KRANZ, P. 1919 Betrachtungen über den Zahnstein. Deut. monatschr. Zahnh., 37, 8–18.

(75) KRONFELD, R. 1933 Histopathology of the Teeth. Lea & Febiger, Philadlephia.

(76) KÜTSING, F. T. 1843 *Phycologia generalis* oder Anatomie, Physiologie und System-kunde der Tange. F. A. Brockhaus, Leipzig.

(77) LEHMANN, K. B., AND NEUMANN, R. 1899 Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik. J. F. Lehmann, Munchen, a. Aufl., Part 2.

(78) LENTZE, F. A. 1938 Zur Bakteriologie und Vakzinetherapie der Aktinomykose. Zentr. Bakt. Parasitenk., Abt. I, 141, 21–36.

(79) LEVADITI, C., NICOLAU, S., ET POINCLOUX, P. 1925 Sur le rôle étiologique de *Streptobacillus moniliformis* (nov. spec.) dans l'érythème polymorphe aigu septicémique. Compt. rend., 180, 1188–1190.

(80) LEWKOWICZ, X. 1901 Recherches sur la flore microbienne de la bouche des nourrissons. Arch. med. exptl. anat. path., 13, 633–660.

(81) LIGNIÈRES, J. 1924 Nouvelle contribution à l'étude des champignons produisant les actinomycoses. Ann. parasitol. humaine et comparée, 2, 1–25.

(82) LIGNIÈRES, J., ET SPITZ, G. 1902 Actinobacillose. Rev. soc. med. argentina, 10, 5–114.

(83) LIGNIÈRES, J., ET SPITZ, G. 1902 Actinomycosis. Bull. Soc. centr. Med. Vetr., Paris, 20, 487, 546.

(84) LORD, F. T. 1910 A contribution to the etiology of actinomycosis. The experimental production of actinomycosis in guinea pigs inoculated with the contents of carious teeth. Boston Med. Surg. J., 163, 82–85.

(85) LORD, F. T. 1910 The etiology of actinomycosis. The presence of actinomycetes in the contents of carious teeth and the tonsillar crypts of patients without actinomycosis. J. Am. Med. Assoc., 55, 1261–1263.

(86) LORD, F. T., AND TREVETT, L. D. 1936 The pathogenesis of actinomycosis. Recovery of actinomyces-like organisms from the normal mouth. J. Infectious Diseases, 58, 115–120.

(87) LYONS, C., OWEN, C. R., AND AYERS, W. B. 1943 Sulfonamide therapy in actinomycotic infections. Surgery, 14, 99–104.

(88) McKENTY, F. E. 1913 A study of cases of actinomycosis. Am. J. Med. Sci., 145, 835–857.

(88A) MACKENZIE, I. 1931 Pathogenic Leptothriceae in, A System of Bacteriology in Relation to Medicine. Medical Research Council, London, 8, 91–100.

(89) McWilliams, C. A. 1917 Actinomycosis of phalanx of finger. Annals Surg., 66, 117–118.

(90) Magnusson, H. 1928 The commonest forms of actinomycosis in domestic animals and their etiology. Acta Path. Microbiol. Scand., 5, 170–245.

(91) Magrou, J. 1919 Les formes actinomycotiques du staphylodoque. Ann. inst. Pasteur, 33, 344–374.

(92) Mathieson, D. R., Harrison, R., Hammond, C., and Henrici, A. T. 1935 Allergic reactions of actinomycetes. Am. J. Hyg., 21, 405–421.

(93) Mathis, H. 1938 Der supragingivale Zahnstein. Deut. Zahn-Mund-Kieferheilk., 5, 114–127.

(94) Mendel, J. 1918 Leptothrix asteroide. Compt. rend. soc. biol., 81, 471–475.

(95) Miller, W. D. 1890 Micro-Organisms of the Human Mouth. S. S. White Dental Mfg., Co., Philadelphia.

(96) Moore, B. 1910 The physiological and pathological metabolism of calcium and phosphates in relationship to the deposition of insoluble salts in formation of bones, teeth and calculi. Brit. Dental J., 31, 825–831.

(97) Naeslund, C. 1925 Studies of actinomyces from the oral cavity. Acta Path. Microbiol. Scand., 2, 110–140.

(98) Naeslund, C. 1925 Studien über Speichelsteinbildung. Acta Path. Microbiol. Scand., 2, 244–276.

(99) Naeslund, C. 1926 Studies of tartar formation. Acta Path. Microbiol. Scand., 3, 637–677.

(100) Naeslund, C. 1931 Experimentelle studien über die ätiologie und pathogenese der Aktinomykose. Acta Path. Microbiol. Scand., 8, Suppl. 6, 1–156.

(101) Naeslund, C., und Dernby, K. G. 1923 Untersuchungen über einige physiologische Eigenschaften der Strahlenpilze. Biochem., 138, 497–504.

(102) Negroni, P., and Bonfiglioli, H. 1937 Morphology and biology of Actinomyces israeli (Kruse, 1896). J. Trop. Med. Hyg., 40, 223–232; 240–246.

(103) Neuber, E. 1940 Spezifische Diagnostik und Therapie der Aktinomykose. Klin. Woehschr., 19, 736–741.

(104) Northrop, P. M., and Crowley, M. C. 1943 The prophylactic use of sulfathiazole in transient bacteremia following the extraction of teeth. J. Oral Surg., 1, 19–29.

(105) Parker, F., and Hudson, N. P. 1926 The etiology of Haverhill fever (Erythema arthriticum epidemicum). Am. J. Path., 2, 357–379.

(106) Philipp, H. 1935 Über die Struktur von Zahnstein (Calculus dentalis supragingivalis). Z. Physiol. Chem., 223, 209–214.

(107) Pinoy, E. 1913 Actinomyces et mycétomes. Bull. inst. Pasteur, 11, 929–938; 977–984.

(108) Prinz, H. 1921 The origin of salivary calculus. Dental Cosmos, 63, 231–238; 369–374; 503–510; 619–623.

(109) Puntoni, V. 1937 The relation of Bacillus bifidus (Lactobacillus) and the actinomycetic anaerobes, type Wolff-Israel. Ann. igiene, 47, 157–168.

(110) Puntoni, V. 1939 La classificazione degli attinomiceti (microsyphonales vuill.). Third Intern. Congr. Microbiology, 195–196.

(111) Robin, C. 1847 Des Végétaux qui croissent sur l'Homme et sur les Animaux Vivants J. B. Baillière, Paris.

(112) Robin, C. 1853 Histoire Naturelle des Végétaux Parasites qui croissent sur l'Homme et sur les Animaux Vivants. J. B. Baillière, Paris.

(112A) Robinson, R. A. 1944 Actinomycosis of the subcutaneous tissue of the forearm secondary to a human bite. J. Am. Med. Assoc., 124, 1049–1051.

(113) Rosebury, T. 1944 The aerobic non-hemolytic streptococci. Medicine, in press.

(114) Rosebury, T. 1944 The parasitic lactobacilli. In press.

(115) Rosebury, T., Epps, L. J., and Clark, A. R. 1944 A study of the isolation, cultivation and pathogenicity of Actinomyces israeli recovered from the human mouth and from actinomycosis in man. J. Infectious Diseases, 74, 131–149.

(116) Rosebury, T., and Foley, G. 1939 Experimental Vincent's infection. J. Am. Dental Assoc., 26, 1798–1811.

(117) Rosebury, T., and Karshan, M. 1938 Dental Science and Dental Art. S. M. Gordon. Lea & Febiger, Philadelphia, 250–268.

(118) Rosebury, T., and Waugh, L. M. 1939 Dental caries among Eskimos of the Kuskokwim area of Alaska. I. Clinical and bacteriologic findings. Am. J. Diseases Children, 57, 871–893.

(119) Sabin, A. B. 1941 The filtrable microörganisms of the pleuropneumonia group. Bact. Rev., 5, 331–335.

(120) St. John-Brooks, R. 1931 The aerobic actinomyces. A System of Bacteriology in Relation to Medicine. Medical Research Council, London, 8, 72–78.

(121) Saltzmann, R. A. 1940 Relación del calcio y la actividad fosfatásica de la saliva con la formación del sarro y la velocidad de secretión. Rev. soc. argentina biol., 16, 446–451.

(122) Sampson, W. E. A. 1932 Tristan da Cunba (Surgeon Lt. Commander Sampson's Report). Dental Surg., 29, 219.

(123) Schottmüller, H. 1914 Zur Aetiologie und Klinik der Bisskrankheit (Ratten-Katzen-Eichhörnchen-Bisskrankheit). Dermatol. Wochschr., 58, Suppl. 77–103.

(124) Shahan, M. S., and Davis, C. L. 1942 The diagnosis of actinomycosis and actinobacillosis. Am. J. Vet. Research, 3, 321–328.

(125) Siegel, E. 1935 Aktinomyzeten als Zahnsteinbildner. Arch. Hyg. Bakt., 113, 223–233.

(126) Slack, J. 1942 The source of infection in actinomycosis. J. Bact., 43, 193–209.

(127) Smith, G. H. 1930 Factors affecting the deposition of dental calculus. Australian J. Exptl. Biol. Med. Sci., 7, 45–77.

(128) Söderlund, G. 1927 Die Speichelsteinkrankheit ("Sialolithiasis") und ihr Verhalten zu der primären und duktogenen Speicheldrüsenaktinomykose. Acta Chir. Scand., 63, Suppl. 9.

(129) Steinbach, M. 1935 Agents of Disease and Host Resistance, Gay et al. C. C. Thomas, Springfield, p. 977.

(130) Sullivan, H. R., and Goldsworthy, N. E. 1940 A comparative study of anaerobic strains of actinomyces from clinically normal mouths and from actinomycotic lesions. J. Path. Bact., 51, 253–261.

(131) Talbot, E. S. 1913 Interstitial Gingivitis and Pyorrhea alveolaris. The Ransom & Randolph Co., Toledo.

(132) Tascher, P. J., and Wagreich, P. 1943 Salivary phosphatase, relationship of, to calculus deposition. Senior thesis, School of Dental and Oral Surgery, Columbia University, N. Y.

(133) Taylor, W. E. 1934 Association of bacteria with dental calculus. J. Dental Research, 14, 237.

(134) Tenenbaum, B., and Karshan, M. 1937 Factors in human saliva correlated with the occurrence of salivary calculus. J. Am. Dental Assoc., 24, 1255–1259.

(135) Tenenbaum, B., and Karshan, M. 1939 Factors in saliva correlated with the occurence of calculus. J. Am. Dental Assoc., 26, 1965–1971.

(136) Tenenbaum, B., and Karshan, M. 1944 The composition and formation of salivary calculus. J. Periodontol., In press.

(137) Thjøtta, Th., Hartmann, O., and Boe, J. 1939 A study of Leptotrichia trevisan. History, morphology, biological and serological characteristics. Oslo, 1 Kommisjon Hos Jacob Dybwad, 1–41.

(138) Topley, W. W. C., and Wilson, G. S. 1937 The Principles of Bacteriology and Immunity. Wm. Wood & Co., New York, 2nd ed.

(139) Trevisan, V. 1879 Cited by Buchanan, R. E., General Systematic Bacteriology. Williams & Wilkins Co., Baltimore, 1925, p. 352.

(140) TUNNICLIFF, R. 1926 The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, 38, 366–370.

(141) TUNNICLIFF, R., AND JACKSON, L. 1930 *Vibriothrix tonsillaris*. The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, 46, 12–17.

(142) VICENTINI, F. 1897 Bacteria of the Sputa and Cryptogamic Flora of the Mouth. Baillière, Tindall and Cox, London. Cited by Beust, T. B., 1937 J. Dental Research, 16, 379–386.

(143) WAKSMAN, S. A. 1940 On the classification of actinomycetes. J. Bact., 39, 549–558.

(144) WAKSMAN, S. A. 1941 Antagonistic relations of microörganisms. Bact. Rev., 5, 231–291.

(145) WAKSMAN, S. A., AND HENRICI, A. T. 1943 The nomenclature and classification of the actinomycetes. J. Bact., 46, 337–341.

(146) WALKER, O. 1938 Sulphanilamide in the treatment of actinomycosis. Lancet 234, 1219–1220.

(147) WARREN, J. 1942 Observations on some biological characteristics of organisms of the pleuropneumonia group. J. Bact., 43, 211–228.

(148) WARWICK, W. T. 1923 A clinical contribution to the aetiology of actinomycosis. Lancet 205, 497–501.

(149) WHERRY, W. B., AND OLIVER, W. W. 1916 *Leptothrix innominata* (Miller). J. Infectious Diseases, 19, 299–303.

(150) WILKINSON, E. E. 1941 Actinomycosis treated with sulfanilamide. J. Pediatrics, 18, 805–810.

(151) WILLSMORE, N. M. 1937 A study of the osmotic pressure and viscosity of saliva and their relation to common oral conditions. Australian J. Dentistry, 41, 161–195.

(152) WOLFF, M., UND ISRAEL, J. 1891 Ueber Reincultur des Actinomyces und seine Uebertragbarkeit auf Thiere. Virchow's Arch., 126, 11–59.

(153) WRIGHT, J. H. 1905 The biology of the microörganism of actinomycosis. J. Med. Research, 13, 349–404.

(154) WRIGHT, J. H. 1925 Modern Medicine (Osler, W., McCrae, T., Funk, E. H.) Lea & Febiger, Philadelphia. Chapter 6, 783–798.

(155) ZANDER, H. A. 1941 The distribution of phosphatase in gingival tissue. J. Dental Research, 20, 347–353.

RECENT ADVANCES IN OUR KNOWLEDGE OF THE PHYSIOLOGY OF MICROÖRGANISMS[1]

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About a century ago Pasteur started to develop his thesis that there are a large number of characteristic decomposition processes of organic materials which are brought about by the activities of various microörganisms. As good examples may serve the conversion of sugars to alcohol and carbon dioxide, to lactic acid, to butyric and other fatty acids and gases; the oxidation of ethyl alcohol to acetic acid, etc. For each of these processes a certain microbe or a group of closely related organisms was held responsible.

Most of Pasteur's studies in this field were carried out with cultures which nowadays no one would be willing to consider "pure" in the strict sense of the word. This did not materially affect the development of Pasteur's thesis which has gradually become one of the foundations of our present-day science. That, even with impure cultures, Pasteur could arrive at this fundamental concept is due in part, but only in part, to his keen power of observation. Nevertheless, an important adjunct has unquestionably been the fact that he used to a large extent simple and logically composed culture media for his experiments. This resulted in the rapid development of a microflora which, in many cases, was restricted to a few easily recognizable types.

Pasteur also anticipated that microörganisms play a significant rôle in human welfare, especially as causative agents of diseases. The careful study of these phenomena, soon initiated, proved of such vital importance that in a relatively short time tremendous strides were made in the diagnosis, cure and control of disease. So spectacular were the results that it is fully understandable how this aspect of the activities of microörganisms rapidly attracted nearly all the attention, of students and public alike. To a great majority the study of these creatures became the equivalent of the study of diseases, their cause and cure.

Nevertheless, there were a few scientists, like de Bary, Cohn, Winogradsky, Beijerinck, who, by their investigations of the general aspects of the microbes themselves, kept alive an interest in the more fundamental problems of biology. In recent years it has become clear that this has been fortunate; more and more the evidence points to the benefits that can be derived from the use of these "simple" living beings for the study of the basic problems of life.[2]

At present there is a distinct tendency to recognize this, and to attempt an

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[2] *Cf.*, also, Otto Rahn's statement: "While practically all the discussion in the book refers to bacteria, the principles developed reach beyond the domain of bacteriology, and apply to biology generally. More than that, I believe that some of the principles of biology can be found and studied *only* with the simplest forms of life, and that general physiology has much to learn from the physiology of bacteria." (1, p. viii).

amalgamation of various interests, a new integration of knowledge and viewpoints. The inclusion in the program of a general meeting of our Society of a broad paper on the physiology of microörganisms before a joint session is a definite sign of such a drift.

To the speaker who, for many years, has realized and advocated the advantage of "General Microbiology" this is, of course, gratifying. It involves, however, the difficulty of preparing a presentation which, on the one hand, is simple enough to make the problems intelligible to a general audience, and on the other, includes enough of interest to the various "specialists". In spite of the temptation not to disappoint the latter group, I have decided to concentrate on the former aspect.

As previously stated, Pasteur's approach to the culture of microörganisms was eminently rational. The advent of "medical bacteriology" gradually led to an unfortunate change in this respect. I do not mean to imply that it was irrational to use tissue fluids, blood fractions, meat extracts, physiological solutions, and the like for the culture of bacteria occurring in body tissue. On the contrary, the choice of such ingredients appears entirely logical. But the fact is that media of this type could be used satisfactorily also for growing the great majority of non-pathogenic organisms. And this resulted in the adoption of such complex materials as "standard media" for general use. Thus the culture methods became stereotyped, entailing the regrettable consequence that one of the more important problems of microbial physiology, that of the nutrition of microörganisms, was not even recognized as such by most bacteriologists. It is true that there were isolated instances which clearly demonstrated an awareness of the fundamental principles of nutrition. Nevertheless, it was not until recently that microbial nutrition began to be studied as a legitimate and significant problem. Much of our present knowledge derives immediately from the work of Lwoff (2, 3) and Knight (4).

Just what is it that microbes need as food, in order to enable them to grow, to reproduce themselves? Since not a single case is known in which these creatures accomplish a transmutation of elements, it is obvious that all elements which are part of the living cell must be present in their environment if they shall be in a position to grow. But the form in which these elements are used for the building of new cells differs widely for various organisms.

With the exception of carbon and nitrogen, all such elements can be satisfactorily supplied as inorganic salts. The great diversity occurs with regard to the carbon and nitrogen nutrition. While a great many microörganisms can manufacture all their cell constituents from nitrates or ammonium salts and a single simple carbon compound, such as ethanol, acetic or lactic acid, or others numerous others require additional and more complex substances before they can be made to grow. Some simple considerations will help in clarifying the situation.

As far as has been ascertained, all living organisms are composed of water, carbohydrates, proteins, fats and salts as the quantitatively predominant constituents. In addition they must, in order to function properly, also contain

enzymes, those "biological catalysts" which have been made responsible for most biological activities which reveal themselves in chemical transformations of various sorts, generally referred to collectively as "metabolism". It is then obvious that an organism which can grow, for example, in an inorganic salt solution with ammonium acetate or lactate, must also be able to elaborate all its proteins, carbohydrates, fats, as well as enzymes, from these ingredients. On the other hand, an organism which cannot synthesize an essential protein constituent, such as tryptophane, obviously cannot produce new cells unless it be simultaneously supplied with either tryptophane itself or with a substance from which it can produce tryptophane.

This is quite elementary, as are many problems when properly stated. The great importance of so viewing the topic lies in the immediate clarification of the true nature of the nutritional problems. It was Lwoff who, in 1936, first clearly stated that any substance which an organism cannot synthesize, and yet needs for its developments, constitutes for that organism a "growth factor" (5). This statement appears eminently logical, and, in its ultimate consequences, unavoidable. As a rigorous definition of the term "growth factor" it is, however, not generally adopted; there are various other attempts at defining "growth factors", usually implying that these should be substances of organic nature and required in minute amounts. No matter what special definition or terminology be applied, the concept contained in Lwoff's statement makes it possible to draw some important inferences which have already proved extremely fruitful, as may appear from the following recent developments.

The decisive need for certain specific organic molecules in the nutrition of a microbe implies, on the basis of the above mentioned concept, a, that such compounds cannot be manufactured by the organism in question from other substances and b, that they must play a fundamental rôle in the well-being of the cell which, without a definite supply of these materials, cannot function normally. Where this mode of reasoning is applied to those growth factors needed in minute amounts, it is, furthermore, necessary to conclude that only a relatively small number of molecules per cell is sufficient to satisfy its needs. This, in turn, leads to the inference that such a substance, the qualitative requirement for which appears so much out of proportion with its absolute importance, must fulfill an extremely active rôle. In this manner we are led to search for a direct connection between this group of growth factors and highly active cell constituents—that is, enzymes. If the particular organic molecules were needed for the manufacture of enzymes, then both the very small amounts required, and the vital necessity would be accounted for.

Here it may be recalled that the first chemically recognized enzyme constituents, riboflavin, thiamin, and nicotinic acid or its amide, were almost simultaneously identified as specific growth factors for certain microörganisms. And these are also substances which have been known as vitamins for the higher animals.

There are a number of cases where a fundamental connection if not identity between such growth factors for microörganisms and typical vitamins has been

revealed.   In fact, it is an easily defensible thesis that all vitamins for metazoa will have their counterparts among the microbial growth factors. This, of course, strengthens the view that, in its basic features, the metabolism of all living organisms is fundamentally the same.   And thus studies on growth factors for bacteria, fungi, and protozoa are of importance not only for the acquisition of a better understanding of the nutritional problems of microorganisms *per se*, but also for the prospect of the discovery of as yet unknown vitamins. Furthermore, those microbes which display an absolute requirement for specific vitamins have already proved extremely useful and inexpensive tools for the purpose of vitamin assays (6).

One further implication of the interrelations between certain growth factors, vitamins, and enzymes is that those microorganisms which can develop in an environment devoid of one or more of such substances must be able to synthesize them from other foodstuffs. Wherever this has been tested experimentally, it has been found to be true; as has also the conversion of these materials into enzymes in the relatively few cases in which such demonstration has been practicable.

In the foregoing paragraphs a deliberate attempt has been made to discuss the nature and function of specific growth factors in the most elementary sense. Not always are the experimental results quite as clear-cut or simple as those here considered.   It occurs not infrequently that the development of a microbe, while not strictly dependent upon the availability of some special substance, can be greatly furthered by its presence.   The common practice of referring to this type of compound as "growth-promoting factors" has tended to obscure the fundamental concept of the nutritional problem. This is, however, not necessary because results of this kind can be satisfactorily interpreted in line with the earlier developed hypothesis. It may readily be assumed that an organism which shows such a response is intrinsically capable of performing the synthesis of the related cell constituent from other nutrients, but that this occurs at so low a rate that the over-all phenomenon of growth becomes limited by this particular synthetic process.   Hence the presence in the medium of preformed molecules of the compound in question eliminates the necessity for the slow synthesis and consequently can result in an acceleration of growth, now primarily restricted by synthetic processes which take place at a considerably greater rate.

While this phase of the study of microbial nutrition has thus yielded results and concepts which seem clearly to establish the relation between certain kinds of growth factors and vitamins, it cannot yet be asserted that the connection between these substances and special enzymes has been demonstrated equally satisfactorily. This, however, is undoubtedly the result of a still very limited knowledge of the specific chemical nature of the enzymes; the pursuit of growth-factor studies has considerably exceeded that of the chemical composition of enzyme systems.   The chief reason for this lies in the difficulties connected with the purification of enzymes.   Even the preparation of crude but active solutions has, in many cases, proved far from simple.

Nevertheless, advances have been made in this respect, in part due to the introduction of new techniques. Among these the grinding mill of the English workers (7) and the powdered glass technique of Werkman *et al.* (8) are especially worthy of note. It becomes increasingly clear that a satisfactory picture of metabolism must entail a more penetrating comprehension of the chemical nature of these processes. And in view of what has been achieved in a few isolated cases, it is not too much to hope that this will ultimately lead to a fine resolution of the over-all changes of foodstuffs to excretory products and production of cell constituents into a series of simple steps, each proceeding under the influence of its particular and special enzyme system.

The few general principles in this field which have been developed in the past 25 years, and which have proved so eminently fruitful in guiding our future progress, have above all comprised the concept that any biochemical process consists of a more or less extended series of step-reactions, proceeding in order, and each one step representing a simple, chemically intelligible reaction. If the primary substrate consists of complex carbohydrates, oils or fats, or proteins, it was considered probable that these would undergo a preliminary breakdown by hydrolysis. The split products would then be subject to a series of further breakdown reactions the nature of which is in essence a hydrogen transfer. Hereby the original substrate is either oxidized, or it may be split into smaller units, while other substances or some of the split products are simultaneously reduced.

Various objections have been raised especially against the assumption of a preliminary hydrolysis of di- and polysaccharides. A number of experiments have indicated the existence of microörganisms which are able to decompose some polysaccharides but which apparently do not attack the constituent hexose units. A careful study of such cases has, without exception, shown that the interpretation of the experimental results was at fault. One of the most persistent claims, pertaining to the decomposition of cellulose by a group of bacteria unable to utilize glucose, was resolved recently by Stanier (9) who emphasized once again the long known but often forgotten production of toxic products during the heat-sterilization of glucose solutions. There is at present not a single authenticated instance on record of microörganisms which can attack some polysaccharide but not its hydrolytic products.

There are, however, some well-verified examples of organisms decomposing di- and polysaccharides faster than the simple hexose constituents. Clearly this could not be so if the supposed hydrolyses initiate the decomposition of the polymers. The elucidation of this situation has resulted from the application of the discovery, by Cori (10), that muscle tissue depolymerizes glycogen with the production not of glucose but of glucose-1-phosphate, the now famous Cori-ester. The greatest importance of this discovery lies in the fact that this type of reaction is enzymatically controlled, and reversible, thus making possible the enzymatic synthesis of glycogen or starch from glucose-1-phosphate. This concept was applied by Doudoroff to the decomposition of sucrose by *Pseudomonas saccharophila*, which proceeds considerably faster than that of glucose,

fructose, or invert sugar (11). A spectacular but logical outcome of this investigation has been the experimental demonstration of an enzymatic synthesis of sucrose, not from glucose and fructose, but from Cori-ester and fructose, again as a completely reversible process (12).

While it would be premature to assert definitively that all decompositions of di- and polysaccharides proceed by way of such "phosphorolyses" rather than hydrolyses, it appears, nevertheless, as a most logical and attractive working hypothesis. This manner of depolymerization leads directly to products which function as the first intermediate stages in the gradual breakdown of the simple sugars.

It should be emphasized that these recent developments in an understanding of carbohydrate breakdown imply far more than the replacement of a theory, gradually shown to be inadequate, by one which is at present more satisfactory; they also pave the way towards a deeper insight into the synthetic processes in metabolism.

For a long time physiologists have been more or less satisfied with the concept that metabolism consists of two fundamentally opposite processes: catabolism and anabolism, or breakdown and synthesis. These two phases were considered as energetically coupled; the synthetic processes, requiring energy, were made possible by the occurrence of catabolic reactions in which energy is liberated.

But the successful interpretation of the more intimate mechanism of catabolic processes has inevitably led to the desire to approach biological syntheses in a similar manner, and to comprehend the precise chemical nature of such reactions. It is obvious that the conversion of lactate and ammonia into carbohydrates, proteins, fats, nuclear materials, enzymes, etc., is ultimately chemistry in the same sense as is the conversion of sugar to alcohol and carbon dioxide, or to lactic acid. The only real difference is that the former conversions are far more complicated. Now, the first examples of an enzymatic synthesis of carbohydrate show an important principle: it is not the hexose itself but a product, arising therefrom in the course of the gradual breakdown, which functions as the immediate raw material from which a spontaneous, enzymatic synthesis can proceed.

A very similar situation has been known for some time in the case of the synthesis of amino acids. This has been achieved by the addition of ammonia to a keto acid or to an unsaturated acid with the subsequent reduction of the imino acid. Both these reactions have been recognized as reversible and enzyme-controlled, (13). Recent evidence to the same effect has come from the studies of Bonner et al., which have shown that mutant strains of *Neurospora*, unable to synthesize leucine, isoleucine, and valine from glucose and ammonium salts can do so if supplied with the keto-acid analogs of these amino acids (14).

We are, therefore, in possession of a number—a very small number, it is true— of examples which show that synthetic reactions, polymerizations as well as the formation of new linkages, are comprehensible as enzyme-controlled, simple chemical processes. Also in this category belongs the synthesis of four-carbon compounds from three-carbon compounds by the addition of carbon

dioxide to a three-carbon molecule, discovered by Wood and Werkman (15). This type of reaction is now recognized as of extremely wide-spread occurrence (16). One important aspect of these syntheses must again be stressed: the initial components for such reactions comprise in part not the original food constituents but conversion products thereof. And this group of products, *e.g.* Cori-ester, keto acids, etc., arise generally and readily during the gradual breakdown of the original substrate.

Following this trend of thought, it becomes rational to look upon the biological syntheses as the result of series of consecutive step reactions for which one or more of the reactants are furnished immediately by catabolic processes inasmuch as they represent intermediate products in the chain of individual steps comprising the breakdown of the substrate.

This is also the place to call attention to the eminently important studies which have led Lipmann and others to a new concept connected with the gradual degradation of oxidizable materials by dehydrogenations (17, 18). Formerly it was considered probable that, whenever a double bond originated by dehydrogenations—as, for example, in the formation of an aldehyde, keto acid, or unsaturated fatty acid—the addition of water as H and OH to the double bond would precede a further dehydrogenation. It now appears likely that, in analogy with the addition of phosphate during phosphorolysis of polysaccharides, also here not H_2O but phosphate is added. The subsequent oxidation of such phosphorylated products then leads to the formation of curiously unstable, that is highly reactive, substances from which a number of syntheses may be expected which it would be impossible to achieve with the phosphate-free molecules. And it is of great significance that Vogler and co-workers have shown that even during the oxidation of an inorganic substrate, *viz.* sulfur, by the autotrophic bacterium *Thiobacillus thiooxidans* such labile, active organic phosphate compounds arise, especially since it has further emerged that these substances are intimately linked with the synthesis of cell materials from carbon dioxide (19). That it is very probable that also in the photosynthetic carbon dioxide assimilation such phosphorylated substances participate can only be mentioned in passing (see, for example, 20); suffice it here to emphasize the very general importance of phosphorylated organic compounds for the problem of biological syntheses.

Hence it appears that a profound study of the steps involved in the breakdown reactions will do more than yield a better insight into the mechanism of just these processes. It would furnish ever more complete information as to the exact chemical nature of the various intermediate products, and thus also tend to reveal the steps by which biological syntheses proceed. It is particularly in this sense that studies such as Foster's on the microbial decomposition of riboflavin must be understood; they aim ultimately at the elucidation of the mechanism of its biological synthesis (21).

But it is not only a study of catabolic reactions which can supply important information concerning the mechanism of anabolic reactions. Other approaches are possible, and one, in particular, has contributed some beautifully clear results.

In principle it rests on the following consideration. An organism may be unable to develop in a simple medium because it lacks the ability to synthesize a specific component of its normal cellular constituents. When, therefore, the organism is inoculated into the simple medium enriched with this substance, growth will occur. By supplying, instead of the compound itself, such materials as may be deemed probable precursors in its biosynthesis, the experimenter can deduce from the growth response of the organism which substance or substances are stages in the elaboration of the specific cell constituent. It will be clear that the previously mentioned demonstration of the formation of certain enzymes from vitamins is a case in point. Here, however, the details of the biosynthesis are still obscure. Much better examples are furnished by the synthesis of thiamin from the corresponding thiazole and pyrimidine moieties, and of biotin from pimelic acid (3, 22).

Experimental demonstrations of this kind depend upon the availability of organisms known to lack a specific synthetic function, and the literature pertaining to this subject contains a number of examples of such organisms. But until recently this experimental material was restricted to chance isolations. The purposeful production by Beadle and Tatum of special mutant strains of microörganisms, especially of *Neurospora crassa*, by x-ray or ultraviolet irradiation of vegetative cells or spores, has brought about a most auspicious change in this regard (23). Their studies have made available an intensely interesting collection of cultures the members of which are characterized by the inability to synthesize one of a variety of cell constituents. With this material the biosynthesis of arginine from citrulline, and of the latter from ornithine has been shown conclusively (24). The elucidation, by Tatum and Bonner, that the biosynthesis of tryptophane does not proceed from the corresponding keto acid and ammonia, but from a direct junction of indole and serine has also been made possible by the use of these mutants (25). This last investigation is therefore so important because it reveals a new type of synthetic reaction in biological systems which is likely to have a far more general significance for an understanding of the mode of formation of alkyl-substituted aromatic compounds.

The importance of microörganisms for the study of biochemical problems cannot be overemphasized. It is among them that one meets with the almost unlimited diversity of biochemical peculiarities which offers the greatest opportunity for selecting the ideal material, and in its simplest form. It is unnecessary to go into details; it may suffice to mention the existence of organisms which cause the gradual breakdown of some substrate in such clear-cut stages that any one can be isolated and studied separately, as well as of organisms which lack the capacity for synthesizing one single, essential cell constituent from the components of a simple medium, and with which, therefore, precursor studies can so effectively be initiated.

Science is characterized by both a gradual approach and integration, and by a constant attempt at re-defining "ultimate causes"; or, in other words, by a continued search for a more and more detailed explanation.

Where once "miasma" served as an adequate designation of the cause of certain

diseases, it was, after the great work of Pasteur and Koch, more satisfactorily replaced by "specific microörganisms". Later a still deeper penetration became possible. While mediately the bacterium, fungus, or protozoan still remained the "cause", it was the production of a toxin, the destruction of erythrocytes, etc., which more immediately was held responsible.

In the biological sciences, it is one of the present trends to view the events of life in terms of comprehensible chemical processes. The studies of vitamins, hormones, chemotherapy, the newer developments in experimental embryology and genetics, these all show conclusively how great the advance has been. And to the student of microörganisms, it is gratifying to note how impressive a rôle these minute living creatures have played in those developments.

What the future may bring, "who dares predict?" At present it seems inevitable that for a while the field of microbiology will draw an increasing number of students who will devote themselves to the investigation of a number of the fundamental aspects presented by this vast material with its inexhaustible potentialities. And it is likely that there will always be a few, at least, who will continue to do so after the majority has lost sight of the basic problems which the microbes will always offer.

REFERENCES

1. RAHN, OTTO. 1932 Physiology of Bacteria. Blakiston's, Philadelphia.
2. LWOFF, ANDRÉ. 1932 Recherches biochimiques sur la nutrition des Protozoaires. Masson & Cie., Paris.
3. LWOFF, ANDRÉ. 1938 Les facteurs de croissance pour les microörganismes. Ann. inst. Pasteur, 61, 580–617.
4. KNIGHT, B. C. J. G. 1936 Bacterial Nutrition. Med. Research Council, (Britain) Special Rept. Series, No. 210, H.M.S. Stationery office, London.
5. LWOFF, ANDRÉ. 1936 Etude sur les fonctions perdues. Ann. Fermentations, 2, 419–427.
6. WILLIAMS, R. J., et al. 1941 Studies on the vitamin content of tissues. I, II. Univ. Texas Pub., No. 4137; No. 4237, 1942.
7. BOOTH, V. H., AND GREEN, D. E. 1938 A wet-crushing mill for microörganisms. Biochem. J., 32, 855–861.
8. WIGGERT, W. P., SILVERMAN, M., UTTER, M. F., AND WERKMAN, C. H. 1940 Preparation of an active juice from bacteria. Iowa State Coll. J. Sci., 14, 179–186; (see also J. Bact., 42, 665–676, 1941; J. Biol. Chem., 138, 35–48, 1941; Biochem. J., 36, 485–493, 1942).
9. STANIER, R. Y. 1942 The Cytophaga group: a contribution to the biology of Myxobacteria. Bact. Rev., 6, 143–196. See also: Soil Sci., 53, 479–480, 1942.
10. CORI, C. F. 1941 Phosphorylation of glycogen and glucose. In: Biol. Symposia, Vol. 5, 131–140.
11. DOUDOROFF, M. 1940 The oxidative assimilation of sugars and related substances by Pseudomonas saccharophila. Enzymologia, 9, 59–72.
12. DOUDOROFF, M. 1943 Studies on the phosphorolysis of sucrose. J. Biol. Chem., 151, 351–361.
13. VIRTANEN, A. I., UND TARNANEN, J. 1932 Die enzymatische Spaltung und Synthese der Asparaginsäure. Biochem. Z., 250, 193–211.
14. BONNER, D., TATUM, E. L., AND BEADLE, G. W. 1943 The genetic control of biochemical reactions in Neurospora: a mutant strain requiring isoleucine and valine. Arch. Biochem., 3, 71–91.
15. WERKMAN, C. H., AND WOOD, H. G. 1942 Heterotrophic assimilation of carbon dioxide. Advances in Enzymology, 2, 135–182.

16. KREBS, H. A.   1943   Carbon dioxide assimilation in heterotrophic organisms.   Ann. Rev. Biochem., 12, 529–550.

17. LIPMANN, F.   1941   Metabolic generation and utilization of phosphate bond energy. Advances in Enzymology, 1, 99–162.

18. KALCKAR, H. M.   1941   The nature of energetic coupling in biological syntheses. Chem. Rev., 28, 71–178.

19. VOGLER, K. V., et al.   1942   Studies on the metabolism of autotrophic bacteria; I, II, III.   J. Gen. Physiol., 26, 89–102; 103–117; 157–167.

20. RUBEN, S.   1943   Photosynthesis and phosphorylation.   J. Am. Chem. Soc., 65, 279–282.

21. FOSTER, J. W.   1944   Microbiological aspects of riboflavin.   J. Bact., 47, 27–41.

22. EAKIN, R. E., AND EAKIN, E. A.   1942   A biosynthesis of biotin.   Science, 96, 187–188.

23. BEADLE, G. W., AND TATUM, E. L.   1941   Genetic control of biochemical reactions in Neurospora.   Proc. Natl. Acad. Sci., U. S., 27, 499–506.

24. SRB, A. M., AND HOROWITZ, N. H.   1944   The ornithine cycle in Neurospora and its genetic control.   J. Biol. Chem., 154, 129–139.

25. TATUM, E. L., AND BONNER, D.   1944   Indole and serine in the biosynthesis and breakdown of tryptophane.   Proc. Natl. Acad. Sci., U. S., 30, 30–37.

THE SIGNIFICANCE OF THE BACTERIA AND THE PROTOZOA OF THE RUMEN OF THE BOVINE[1,2]

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CONTENTS

One of the most interesting chapters of biology, and probably one of the most neglected in instruction in biology, is that which considers the symbiotic relationship existing between living forms. The term "symbiosis", by derivation, means living together and usually carries the implication that each unit concerned in the relationship derives benefit therefrom and is necessary to the continued existence, in nature, of the associated units. For an informative discussion of the use of the word "symbiosis", the reader is referred to Calkins and Summers (1941, p. 890). The spatial arrangements are never included in the specifications but, by usage, the term is commonly restricted to those forms living in such intimacy that, so far as space is concerned, the associated forms comprise a unit, a family.

The symbiotic relationships existing between members of the world of microbiology and those of the world of macrobiology are among the most interesting and possibly the most important in this complex field of biology. This subject has been the field of interest of many students whose observations have been summarized by Paul Buchner (1930). Five of its 900 pages are devoted to the phase of which this paper is a part; namely, the relationships existing between the mammals and their associated microflora and microfauna. These relationships appear in the most evident form between the higher animals and the bacteria and protozoa of the alimentary tract, and especially between the polygastric animals and the flora and fauna of the upper part of the alimentary tract. If it is at all possible to arrange the symbiotic relations between mammals and their microassociates in the order of their relative importance to man, it is certain that those between the ruminants and the microörganisms would rank first. Yet less than one page of Buchner's review and fewer than 12 of the approximately 1250 references relate to this subdivision of the subject.

The subject has also been reviewed by Scheunert (1925), and by Scheunert and Schieblich (1927), and still more recently by Schwartz (1935). The latter review does not include a discussion of the mammals and their symbionts.

Kirby has reviewed the relationships between certain protozoa and other animals in "Protozoa in Biological Research" by Calkins and Summers (1941). Fourteen pages are devoted to the ciliates of ruminants.

It is evident that the symbiosis of mammals and microörganisms has been studied much less than has that between the lower animals and the microörganisms. The explanation is evident: it is much easier to work with insects than with mammals.

I. THE RUMINANT

The most important to man's economy of the symbiotic relationships between mammals and microörganisms are found in the ruminants, the cud-chewing animals, in which the lower part of the esophagus forms a large sack, so large indeed that the food can be held therein for some 24 hours in the case of the bovine and from which it can be returned to the mouth for mastication. This arrangement permits the animal to ingest its food rapidly and to chew it thoroughly at a later time, a condition of great advantage to the animal in its feral state since during grazing it is more exposed to attack by its carnivorous enemies than at other periods. Hence the shorter the time that is required for the taking of food, the more likely it is to escape destruction. This arrangement also enables the ruminant to utilize certain kinds of energy-containing compounds to a much greater extent than can the non-ruminating herbivore. In most parts of the world the continuous growth of plants is not possible, due to sequence of seasons or to the alternation of wet and dry periods. Thus at intervals the herbivores must live on vegetation that has completed its cycle of growth, that has become dry, woody and resistant to the agents that degrade it into the simple products that can be absorbed from the alimentary tract. The species that can obtain the greatest amount of energy and nutriment from each unit of such material will not suffer the same handicap as will those that can obtain less benefit from it.

These two conditions seem to account for the fact that the ruminants predominate among the larger mammals in all parts of the world. They include all the cloven-hoofed animals other than those of the swine group. They are the most important that man has brought to his use as sources of power, clothing, and food. The world without the cow, the sheep, the goat, the camel, the water buffalo, the llama, the yak and the reindeer would be far different than it has been or is. Without domestic and wild ruminants nothing like the present population of the world could be fed, for a large part of the yearly crop now used to produce food for man would be returned to the soil as it is formed by the plant, or it would be used by organisms that cannot be eaten by man or, at least, are not now so consumed. The greater part of the organic matter built by the green plant consists of carbohydrates. The simplest and most soluble of these, the sugars, can be used by man and all the higher animals. The somewhat more complex and insoluble starches can be used by man and by most higher animals. These classes of compounds make up but a small part of the mass of matter constructed by the green plant. The more complex celluloses and lignins, wood

substances, are much more abundant and are used by only a part of the higher animals.

Before any of the carbohydrates occurring in nature in any significant amounts can be used by the animal they must be so changed that they can pass into the blood stream and be utilized by the tissues. These changes are occasioned primarily by the various enzymes which are formed in the tissues and then excreted into the alimentary tract there to carry on their work of preparing the ingesta for absorption. No one of the mammals is known to produce an enzyme that will change cellulose and lignin into soluble compounds, but among the microbial associates of many animals are found, in some part of the digestive tract, forms that can produce enzymes that attack these compounds and prepare them for absorption. It is difficult or impossible to demonstrate these enzymes, as those that act on starch can be demonstrated in the liquid of the tract. It may be that the action is due to the intimate contact of the living cell with a particle of the compound, and that in some manner the material at the point of contact is made soluble in the menstruum at that point and thereupon pass into the cell. This has been demonstrated by Vogler and Umbreit (1941) for sulphur which is used by certain of the bacteria of the soil and by this action is made available for other forms of life.

It is commonly supposed that the enzyme is formed in excess of the needs of the microörganism and that the excess of the decomposition products, such as glucose from cellulose, is then available for the animal. It seems more likely that the action is so largely a contact phenomenon, that the host has opportunity to use these compounds coming from the degradation of cellulose by the microorganism only in the form in which they are built into the microbial cell. The result is the same so far as the host is concerned, for in either case whether the enzymes are of intrinsic origin or are formed by bacteria the material ingested is prepared for absorption.

It is commonly supposed that a larger part of the energy of the food is available to the animal when the enzymes are formed by it rather than by some other form that is living on the ingesta. It may be, however, that it makes little difference in the energy relations where the enzyme is elaborated. The animal and its associates may comprise a true digestive or physiological unit.

1. *The rumen and its rôle.* The term "rumen" as used in this paper includes the rumen proper or paunch, the reticulum or honeycomb, and the omasum or manyplies, the latter names giving some idea of the structure of these organs. The rumen is by far the largest of the three. The essential rôles of the other sacculations of the esophagus are not well understood and hence it seems proper to class all together so far as our discussion goes. The rumen of the bovine is slightly developed at birth. It slowly increases in size relative to that of the true stomach under the stimulus of solid food, especially of the type available to the animal in its native state. Such material in modern terminology is called roughage. The rumen will develop in the absence of such material, as for example, when only ground grain is fed which would contain only a small amount of cellulose and lignin. The writer has seen no statement as to whether the

rumen will develop if only milk is consumed. It has been stated that it will be reformed if removed by operation. In the adult bovine, the capacity of the rumen is such that when filled its contents will make up about one-fifth of the total weight of the animal. The rumen of an animal weighing 1000 pounds has a capacity of about 25 gallons.

The size of the rumen and the fact that the liquid therein contains no enzymes elaborated by the animal have caused students of the nutrition of the ruminant to look on the rumen as a storage chamber in which the food is kept until there is opportunity for its proper mastication and salivation which prepare it for digestion in the glandular stomach and in the lower levels of the tract. This view of the rôle of the rumen is still prevalent as is reflected in the following quotations from the volume entitled "Nutritional Physiology of the Adult Ruminant" by Ritzman and Benedict, (1938): "The paunch is a large warm storage vat that secretes no digestive juices", p. 7; "The function of this organ is of a purely mechanical character", p. 20; "That the greater efficiency of the ruminant in digesting coarse fodder is due to provisions of a mechanical rather than of a chemical nature appears to be well demonstrated experimentally", p. 8.

The incorrectness of this view is at once evident to one who views a drop of the liquid from the rumen under high magnification and thus is made aware of the abundance of microscopic forms living in the rumen. The protozoa and bacteria represent a density of population that is never met in vitro and probably not elsewhere in nature. This population has arisen from the material ingested by the animal and to think that this microbial population has no rôle in the economy of the animal is possible only for one who has had no training in microbiology. It would seem that much of the research and some of the feeding practices would never have arisen had the viewpoint of the microbiologist been considered. For example, until recently it had been thought impossible for the ruminant to care for any part of its need for nitrogen from urea, a waste product of the metabolism of the animal. It is now known that such is possible. The bacteriologist could have answered the question from his knowledge of the ability of many bacteria to utilize this source of nitrogen in the presence of appropriate sources of energy, and from his knowledge of the abundance of bacterial growth in the rumen. But he could not have answered the question as to the part of the total needs that could be derived from such a source.

2. *Conditions in the rumen.* W. B. Cannon has discussed in his book "The Wisdom of the Body"(1932) the ways and means by which a condition of constancy is maintained by the body in its various parts. If one considers the rumen a true organ, he would expect to find therein the same constancy characteristic of other organs. It is evident that the temperature therein will be that of the species in question, in the bovine about 39 C (102 F). This will aid in maintaining a constant type of microbial population. The usual food of the ruminant is such that the carbohydrates from which acids are formed by bacteria are in greater abundance than are the proteins from which alkaline substances are formed. Hence the reaction of a fermenting mass of plant material is likely to become acid in reaction, so acid indeed that further growth of

the bacteria is impossible, as in such self-pickled products, silage and sauerkraut. The same inhibiting action would take place in the rumen were there not provisions to prevent it.

The saliva of the bovine carries no enzymes. It does contain about 0.9 per cent of solids of which over one-half is inorganic matter, largely sodium bicarbonate. Man secretes about 10 ml of saliva per pound of body weight per day; and the fowl about 4 ml. The bovine secretes about 45 ml of saliva for each pound of live weight per day. Thus an animal weighing 1000 pounds secretes about 45 liters each day or about 90 pounds. The saliva has a pH of about 8.2. The neutralizing power of this great volume of saliva is evident By this means the reaction of the rumen is kept at a point that is favorable for the growth of bacteria and protozoa, just below pH 7. It seems probable that the ingestion of acid food such as silage causes an increased flow of saliva so that the constancy of environment so essential to the proper functioning of any organ is maintained. The saliva has additional rôles such as the lubrication and moistening of the food both at the time of its ingestion and later when it is cudded.

The oxidation-reduction conditions are such that only anaerobic organisms or the more anaerobic of the facultative ones can grow. Under the anaerobic conditions at which growth must take place in the rumen, there is very little release of energy as heat in spite of the enormous transformation of matter that is taking place. This loss is inconceivably small to one accustomed to thinking in terms of aerobic processes.

The wall of the rumen is constantly passing through a series of rhythmic contractions and expansions of such a nature that the food follows a rather definite path, at least any particular kind of food such as dry fodder tends always to follow the same path. The contraction of the walls of the rumen produce a constant mixing of the contents thereof and cause the lighter parts that are found in the upper part of the rumen above the level of the liquid to be constantly sprayed with the liquid, thus tending to remove to the lower layers all small particles and any part of the solid made soluble. A very large volume of gas is formed, which as it passes to the upper part of the rumen aids in the mixing of its contents. This gas is passed to the exterior through the esophagus and if, for any reason, the opening thereof becomes clogged a condition known as bloat or hoven results, which if not relieved causes the death of the animal. Many of the microörganisms of the rumen are motile and aid in the mixing of its contents. The result is that no part of the contents becomes stagnant and that the environment is controlled throughout the rumen.

The resultant of all of these factors of control is an environment constantly favorable to the reproduction and persistence of certain protozoa and bacteria. Fresh supplies of food are arriving at more or less regular intervals. Irrigation by saliva and water maintains a constant reaction and removes the products formed, as well as a part of the microörganisms, to the lower levels of the rumen from which they pass into the lethal environment of the glandular stomach, there to be digested and made ready for absorption. The transformation of

food into microbes is so great that some have been led to say that the ruminant lives not on the material ingested but upon the mass of microörganisms that has been formed from that material. This statement is certainly more nearly true than one would infer from the modern literature on the digestive processes of the ruminant.

3. *The esophageal groove.* The lower part of the esophagus is a slit tube. The edges of the slit can be brought together if the food is to be passed to the omasum and thus to bypass the rumen proper; or the slit may be opened, in which case the food will enter the rumen there to be exposed to changes that will decrease the energy content from that which the food would have had if it had escaped such exposure. The very young animal lives exclusively on milk which can best be utilized if it passes directly to the true stomach. All seem to admit that the groove is under the control of the young animal and that the degree of control will depend on the position of the head as the milk is taken, being greatest when the head is in the suckling position and less perfect when in the drinking position. Thus some advocate that calves be fed from an artificial udder rather than from a pail. It also seems that the rapidity with which the milk is taken is of significance. If it is taken slowly, it will bypass the rumen, while if it is taken rapidly, much of it will enter the rumen.

Schalk and Amidon (1928) from a long series of observations on both young and adult bovines with artificial fistulae in the rumen conclude that the adult has little control over the esophageal groove and that saliva, water and all food enter the rumen. Ritzman and Benedict (1938, p. 288) state that in the case of the adult bovine only mucus is passed by the rumen and reticulum to the abomasum. They also state that concentrates such as meal do not enter the rumen. It is to be remembered that the domestic bovine ingests much material of a nature quite unlike that which it ingests in a wild state. It now receives considerable quantities of grain, either ground or unground. It seems clear that, while such finely divided material may enter the rumen, it remains there but a relatively short time, for the time any material remains in the rumen seems to depend on its density, heavy material being passed on much more rapidly than the lighter and more fibrous material that forms the main bulk of the food for the wild ruminant. The entrance of the food into the rumen or its bypassing thereof is important; so also is the time it remains therein. The shorter the time, the less will it be robbed of energy by the microbial process to which it is exposed.

It is evident that saliva must be passed into the rumen since otherwise it could not exert its buffering action, its most important rôle. The bolus swallowed after rumination is returned to the rumen but apparently quickly passes to the omasum.

Water taken in large quantities must either bypass the rumen or remain therein but a short time. The liquid passing out of the rumen must tend to carry with it the soluble matter and also that most finely divided, the extreme of which will be the bacteria. This is indicated by the observations of Mills et al. (1944) regarding the conditions under which urea is used by the bovine.

The results tend to show that starch is more effective than sugar in the form of molasses as a source of needed energy if urea is to be changed to protein. This difference may be due to the fact that the starch is retained in the rumen and becomes a source of energy for the bacteria that are building protein from the urea while the sugar passes beyond their reach with such rapidity that they cannot make full use of it.

The average loss of digestible energy, or that part of the total energy of the food which is used by the animal, in the form of methane produced in the rumen is, according to Ritzman and Benedict (1938), 11 per cent when a diet of hay, grain and silage is used. It is probable that hydrogen is formed by bacteria from carbohydrates in the rumen, and that in the environment of the rumen CO_2 is reduced by the hydrogen to form CH_4. Apparently not all of the hydrogen is thus combined at all times, so it is found occasionally in the gases of the rumen.

Other energy-containing gases are also produced in small amounts, such as hydrogen sulphide and carbon monoxide. These may be absorbed and subsequently excreted in the exhaled air. Some believe they are produced in larger than normal amounts in case of bloat and produce symptoms of toxicity. The addition of elemental sulphur to the feed will cause poisoning through the production of H_2S. It is evident that if the material ingested is of a nature that can be digested in the true stomach, much is gained if it does not pass into the rumen since it will be protected then from this 11 per cent loss. In the case of material such as cellulose, which cannot be handled by the true stomach, the energy made available to the animal will be greater if the matérial enters the rumen than if it does not. From the standpoint of economy of utilization of energy, the animal should have some control of the path of the food. This is apparently more important under domestic than under feral conditions in which the main mass of the food will be best utilized if it enters the rumen.

4. *Rumination.* The content of the rumen is kept in constant motion through the rhythmic contractions of the wall. These must be of such a nature that the various parts of the food will yield the maximum of value to the animal, and all parts of the same class of food will be retained in the rumen for much the same length of time.

At intervals a mass of the fibrous material in the rumen is forced up the esophagus to the mouth where it is masticated for about one minute. The stimulus for the raising of the bolus is apparently the irritation of the wall of a certain part of the rumen by solid, rough feed. It is often stated that the bolus is thus saturated with saliva, becomes heavy and is prepared to pass to the omasum. It is difficult to see how the squeezing to which the bolus is subjected in the chewing can do other than remove water from it with the result that it will become lighter rather than heavier, in spite of which it seems reasonable to suppose that the bolus just ruminated soon finds its way to the true stomach. Otherwise there would be disorder and inequality of treatment. The cud is not from the food most recently ingested, but from that which has been in the rumen for 12 to 24 hours. It has been exposed to the action of bacteria and is probably

so changed that the mastication is much more effective than at an earlier stage. Microscopic examination of the rumen contents shows much more disintegration of the plant tissue than could result from purely mechanical action.

The average weight of each cud or bolus is about 100 grams, the average time of mastication about one minute. Since about one-third of the time is spent in rumination, it follows that about 48 kilos of moist rumen contents are ruminated in each 24 hours, and that the weight of the dry matter involved will be at least half of that ingested in each 24 hours. Dukes (1942) states that a bovine makes about 43,000 motions of the jaw per day in the chewing of the cud. Considerable pressure must be exerted in the process of mastication and the energy demanded must be great. Ritzman and Benedict (1938) estimate it at 11.3 per cent of the total energy in the case of hay.

There are grinding processes at work other than chewing. The constant motion of the contents of the rumen tends to tear the plant tissue apart. The writer has noticed a protozoon swimming in a circle and in each passage rubbing against the particles of food with such force as to cause a distinct depression of its cell wall at the point of contact. Calkins and Summers (1941, p. 979) describe and picture the comminuting action of certain of the protozoa of the rumen. Some seem to tear fibers away. Others bite pieces off. Some ingest large, relative to the cell, flat pieces of tissue and still others take in and roll up large cellulose fibers. Starch is digested and glycogen accumulates in the cell. Cellulose-splitting ability by protozoa of the rumen is questionable, Calkins & Summers (1941, p. 982). The food is also ground between the leaves of the manyplies. One may get some idea of the comminuting effect of these processes by imagining what would happen to cotton and linen cloth kept in a washing machine for 24 hours in the presence of organisms able to decompose the cellulose.

The value of rumination is probably shown in the observations of Mead and Goss (1935), who raised calves from birth to 18 months of age on a roughage-free diet consisting of ground grain. In one comparison a finely ground mixture was compared with the same mixture coarsely ground. The animals ruminated on the coarsely ground feed, but not on the finely ground. The results were opposite to those most would expect in that the average weight of the dry feces represented a higher percentage of the feed consumed in the case of the finely ground than in the case of the more coarsely ground as is shown by the following data:

Dry Feces as Percentage of Feed Consumed

COW NO.	COARSE FEED	FINE FEED	DIFFERENCE
34	21.0	19.7	−1.3
35	18.4	21.8	+3.4
37	19.3	21.1	+1.8
39	20.0	23.2	+3.2
Average	19.7	21.5	

The crude fiber on the average in the feces from the coarsely ground mixture was 21.2 per cent, from the finely ground, 22.9 per cent. Apparently the mastication was more effective than the grinding in making the crude fiber available. It is probable that the mastication was aided by a longer period in the rumen in the case of the more coarsely ground than in the case of the finely ground feed.

It has been shown that the animal masticates the feed more completely if it is silage alone than if it is silage plus corn meal, and that the extent of mastication decreases as the ratio of grain to silage is increased. Here as elsewhere in considering the question of utilization of feed, three conditions are involved: Does some part of a particular ration bypass the rumen? If a part of the feed enters the rumen, is it returned to the mouth for chewing? If a part of the feed enters the rumen but is not returned to the mouth, will the effect of its presence in the rumen for a considerable period be a negative or a positive one so far as utilization is concerned?

It is known that one-fourth to one-half of the whole dry corn ingested is voided in the feces, no use whatever having been made of the grain which is resistant to all the digestive juices of the tract. This can mean only that this part of the grain bypasses the rumen or that it is never returned to the mouth. The significance of size of particle is apparently not well known. For example, to what extent will a corn grain be digested if it is broken into two equally sized pieces which are not masticated? Apparently the digestive mechanism of the ruminant is not as well adapted to the handling of relatively resistant materials, when these escape mastication and treatment in the rumen, as is that of the non-ruminating herbivore. Unground grain and very finely ground grain may be less well utilized by the bovine than that which is just cracked or very coarsely ground. It is evident that the student of feeding faces a most complex problem when he attempts to study the value of any ration.

II. FLORA AND FAUNA OF THE RUMEN

The microörganisms of the rumen of each species are apparently characteristic and are derived from the adults with which the young animal is associated at the time of the development of the rumen. The complex of life in the rumen must vary from one species to another, since the environment is not identical in the different kinds of animals; for example, the reaction of the rumen in the sheep is said to be slightly alkaline rather than slightly acid as in the bovine.

The native flora and fauna are supplemented by those of the food which may on occasion carry a great number of microörganisms. Thus, the writer has found over 50,000 actinomycetes in each milliliter of the rumen liquid of a cow being fed dry fodder. These soil organisms, being aerobic, could not have grown in the rumen and must have been ingested. The writer has also found similar numbers of a film-forming yeast that was ingested with silage. Under certain conditions such an aerobic organism will be growing in the upper layers of silage and will then be ingested in great numbers, and if its real origin is not recognized it will be classed as one of the intrinsic elements of the native flora of the rumen. The writer has also isolated from the rumen a rare form of acid-destroying bacteria which for a period was thought to be an intrinsic part of the flora, since

it was facultative in nature. It was finally found to be carried in the silage in the upper layers of which it was growing. Thus, both water and food may be so heavily laden with microörganisms as to cause confusion in the mind of the analyst of the rumen contents. These adventitious forms are apparently of no significance. It seems probable that many of the forms of life reported as having been found in the alimentary tract are those from the outside rather than intrinsic forms. The appearance of plate cultures made on the usual media of the laboratory gives the impression that most of the forms came from the environment of the animal; much the same picture could be obtained from water, soil or air. The cultural methods reveal but a minute fraction of the total revealed by the microscopic examination of the untreated liquid of the rumen, again an indication that the majority of the forms present are peculiar to that environment. Our inability to cultivate them is due to the lack of knowledge of the chemical environment which permits their growth.

The continued existence of the native population of the rumen is made possible by the fact that the rumen never becomes completely empty and by the further fact that the environment is so mild that even though no growth may be possible for a period, the organisms remain not only viable but of high vitality and respond at once to the arrival of food. The rapidity of this response is shown by the rate at which gas is formed following the ingestion of food. This has been measured by a number of investigators and found to reach the maximum rate within an hour after the food is taken, even when such a resistant material as dry alfalfa hay is taken. The lag so noticeable when the bacteria of the laboratory are placed in contact with a fresh supply of food is striking by its absence.

1. *Protozoa of the rumen.* The most evident organisms revealed by the microscope in the liquid of the rumen are the protozoa, because of their relatively large size and great motility. Becker of the Iowa State College has been the principal American student of the flora of the cow, sheep, goat and horse. He and his associates have studied both the qualitative and quantitative aspects of the protozoa and also their physiological rôle in the economy of the animal.

The rumen fauna appears as soon as roughage is ingested, apparently due to the close association of the young with the adult of its species. After the ruminating stage is reached, each cud brings to the mouth a mass of the protozoa growing in the rumen thus to contaminate all the surroundings of the animal and to assure the quick and easy passage to the rumen of the young animal.

Each species of ruminant is supposed to have its characteristic fauna, some elements of which are found in the rumen of other species but not the entire complex. The report by Becker and Talbot (1927) on their examination of the rumens of 26 cows states that the greatest number of times a single kind of protozoan was found in the series was 23. This does not seem to reflect the probable condition in nature. The history of the animals previous to slaughter was not known and the varied treatment may have influenced the results. Recognizing that any invading mixture of protozoa coming from another animal finds in general the same environment as that from which it came, the conclusion of a characterisitc fauna seems inevitable. *Calkins* and Summers (1941, p. 924)

state "that any termite of a species, wherever obtained, will be found to have the same group of flagellate species. Sometimes one or more flagellates are absent, but uniformity in composition of the fanules is the rule". The quantitative relations may vary so widely as to make the demonstration of each of the intrinsic types at each examination difficult without the expenditure of a great amount of time.

The inherent fauna will be supplemented by that of the feed and the water. These forms cannot grow in the rumen but may persist in the mild environment they there meet. The quantitative relations of the different inherent forms will undoubtedly change with marked changes in the feed as from dry fodder to fresh grass. Apparently no detailed studies have been made of these changes and it may well be doubted whether they are of much significance.

The larger forms of protozoa of the rumen are those that ingest particulate matter such as bacteria and small particles of plant tissue. In every sample feeding protozoa are seen, their waving oral appendages causing currents to flow by the oral groove and thus to bring within their reach the particles of food. The total effect of these currents in the mixing of the rumen liquid must be considerable. Soon after the ingestion of food by the animal, the protozoa can be seen filled with the granular matter they have ingested. Thus, when fresh grass is consumed the cells may appear quite green because of the chlorophyll grains ingested. Some of the larger forms have internal skeletons of silica which, together with other elements of a complex structure, seem strange in an organism classed as a unicellular one. The protozoa of the rumen are anaerobic as is shown by observations made by the writer. ⁄In a hanging drop preparation motility ceases in about 15 minutes, while in a wet mount, a thin film of the liquid between slide and cover glass, the cells remain motile for hours.⁄ In the hanging drop the conditions are aerobic, and in the thin film they are anaerobic except at the very edge of the film.

It is probable that a part of the protozoa of the rumen live on soluble matter that must pass through the cell wall.

The chemical task accomplished by the protozoa of the rumen is wholly unknown. It must be kept in mind that they are anaerobic and, in order to secure the needed energy, must degrade a great deal of material just as is the case with the anaerobic bacteria. It is certain that the byproducts will be complex and will serve as food for other protozoa or bacteria in the rumen, with the result that little energy will be released as heat, and with the further result that a new mass of matter will be formed in the new cells. The quality of the new proteins, carbohydrates and fats is unknown but one may well suppose that it is as valuable as the ingested material, if not more so.

The number of protozoa may vary with the type of feed and with the interval of the feeding cycle at which the examination is made. These organisms are constantly reproducing at the rate of possibly four generations per day and are constantly being removed to the true stomach there to serve as food for the animal. It is difficult to give any numbers per millitliter that will be significant. The numbers reported by a number of observers range from 500,000,000 to one billion

per ml. When one considers their size relative to the bacteria, it is evident that even the lower value given represents the synthesis of a large amount of matter in each feeding cycle. Attempts have been made to determine the total mass of protozoal matter in the rumen at a particular moment. Thus Mowry and Becker (1930) estimate that they make up 10 to 15 per cent of the rumen content, Mangold (1929) 4.4 to 8.7 per cent. Ferber and Winogradowa-Fedorowa (1929) estimate that they make up 5 per cent of the total volume and that they represent 20 per cent of the dry matter in the rumen. If any such values are valid, it is clear that the protozoa must be of great significance in the economy of the animal, especially when it is remembered that 20 pounds of dry feed per day is a fair ration for an animal of average size.

Becker and Everett (1930) carried out digestion experiments with normal sheep and with those that had been made free from protozoa by treatment with copper sulphate. They noted a better utilization of the feed by the latter, a conclusion that has not been confirmed by other observers to the knowledge of the writer. In the absence of protozoa, bacteria would represent the end of the biological cycle in the rumen. The energy of transformation from bacteria to protozoa under the anaerobic conditions of the rumen is probably not great. No one is in a position to say which is the better feed for the bovine, bacteria or protozoa. Nature seems to have determined that the ruminant will live to a large extent on each since both are constantly being carried into the true stomach, there to be killed by the acid and then digested.

No one can say what part of the bacteria is consumed by the protozoa of the rumen. According to Calkins (1938), a single cell of *Paramecium caudatum*, one of the protozoa of the bovine rumen, may ingest two to five million *Escherichia coli* in 24 hours.

Becker (1932) has suggested the following possible rôles for the protozoa in the rumen:

1. Make easily digestible material
2. Harmful
3. Harmless
4. Check growth of bacteria and prevent putrefaction
5. Aid in the utilization of cellulose
6. Aid in mixing the contents of the rumen

They probably do little to check the growth of bacteria since these are undoubtedly always sufficiently numerous to utilize all food available to them or at least when aided by those protozoa that secure their food as do the bacteria by passage through their cell walls. The byproducts of any one form can never accumulate in the presence of such a balanced biological system as that in the rumen. Hence a state of stagnation as is implied by the term putrefaction cannot occur.

The possibility of the rôle given under 5, above, is indicated by the work of Cleveland (1923) who demonstrated the similar rôle of protozoa in the termites.

Most of the detailed work with biological systems has been done under aerobic conditions, as for example, in natural surface waters where the same sequence

of organic matter, bacteria and protozoa is seen.   This cycle continues through the crustacea and the fish.   Juday (1942) has recently emphasized the wastefulness of this cycle.   Thus 97.5 to 99 per cent of the energy of the original material is dissipated by the time the fish stage is reached.   It must be remembered that such a system is operating under aerobic conditions where the transformation of chemical energy to heat is much greater than is true under anaerobic conditions, in which it is difficult to demonstrate the production of heat except when immense amounts of material are being fermented, as occurs in some of the modern industrial plants using anaerobic processes for the production of such products as butyl alcohol and acetone.

   2. *Bacteria of the rumen*.   The microscope reveals the richness of the bacterial life in the liquid of the rumen.   One notes a density of bacteria rarely if ever noted in the cultures of the laboratory.   This is due, of course, to the controlled environment as to temperature, to reaction caused by the buffering effect of the saliva, to the arrival at frequent intervals of fresh food, and to the constant removal of the byproducts of any one kind of organism by others of the complex sequence of life, and through the removal of the cells by the constant stream of saliva and of water passing through the rumen to the lower levels of the digestive tract.   The number of bacterial cells in each milliliter of the liquid of the rumen is expressed by billions.   Some have estimated that 10 per cent of the insoluble matter in the rumen consists of bacteria.

   Cultures from the rumen contents reveal no such numbers as can be seen with the microscope.   One cell out of each hundred or thousand seen can be cultured. This condition has been noted in all studies of the flora of the digestive tract from mouth to anus.   Thus MacNeal (1909) in his extensive work on the fecal flora of man could culture but one, on the average, out of each 3000 cells seen with the microscope.   Similar results have been secured in the study of the numbers of bacteria in the feces of the bovine and of other animals.   The conclusion usually drawn by the bacteriologist, with his implicit confidence in his culture methods, is that most of the cells in the feces are dead.   They must have been formed largely in the lower bowel where no lethal agent for them is known to occur.   It cannot be lack of food, for bacteria rarely die from such a cause, and the great increase in numbers that takes place after the feces are voided is proof that this necessity is not lacking.   This explanation of the marked discrepancy between the numbers shown by the microscope and by any cultural method may seem reasonable to many, but will seem much less reasonable when applied to the contents of the rumen with its inert environment.   The rapid response of the flora to the arrival of fresh supplies of food is proof of the great viability and the high vitality of the bacteria of the rumen.

   A great variety of morphological types of bacteria is seen in the liquid of the rumen, some in great numbers and others in exceedingly small numbers.   One undulating spiral has been seen in every sample examined by the writer; and the examination of scores of fields was, at times, necessary for its discovery.   The great variety of physiological types in each morphological form makes it impossible to decide as to the frequency of occurrence of any one kind by a microscopic

examination. The ability to cultivate only a very small part of those revealed by the microscope also limits the completeness of the picture that the bacteriologist can obtain of the bacteriology of the rumen. The environment of the rumen can be duplicated *in vitro* so far as temperature and reaction at the beginning of the growth *in vitro* is concerned. It seems most probable that the inability of the bacteriologist to cultivate and thus to obtain in pure culture a larger part of the bacteria of the rumen is due to the fact that he cannot create for them the chemical environment they demand. It may be that this environment is provided only by their associates as has been so brilliantly shown by Twort (1913) for one of the pathogenic organisms, that of Johne's disease in cattle, which has been isolated only by the use of a medium that contains an extract of a related organism. As was stated earlier, many of the forms obtained on culture media are identical with those that can easily be obtained from the environment of the animal. Their numbers are small in comparison to the total shown by the microscope. This is evidence that the major part of the flora in the rumen is of types confined thereto, as has been shown throughout the history of bacteriology for a large part of the kinds of bacteria found in the mouth of man. The presence of types that cannot have grown in the rumen, because of the anaerobic conditions therein, indicates again that the present available methods reveal but a most minor part of the types characteristic of the rumen.

The popular idea that the biology of the digestive tract of animals will vary widely with varying food is probably not well based, at least when the normal variations in food are concerned, such as is true with all domestic animals and possibly man. It may be that on such diets as are currently used by students of nutrition in experimental work, the flora may be changed from that which would be present if a normal diet were used. It does not seem probable that, in the ruminant at least, changes in diet will cause changes in the flora of the tract. It would seem that the carbohydrates, the proteins and the fats from different sources are so similar that the biology of the tract will remain relatively unchanged with the changes in diet that normally occur. The corollary of this conclusion is that any class of nutriment will be of much the same value if one admits that it will yield much the same kind of crop of microörganisms in the rumen. On this basis the comparison of different feeds, one of the favorite fields of research of the animal husbandman, seems quite futile.

The estimation of the total quantity of living matter in the rumen at any moment is impossible. The values given earlier may seem to many beyond the realm of possibility. That they are not so unreal as one may think follows from a comparison of the rumen liquid with blood. It is stated, Dukes (1942), that the corpuscles of the bovine blood make up about one-third of its total volume. The number of red cells per milliliter is about six billion, the average diameter is 5.6μ. Thus, in 1 ml. of blood there will be about 396 billion cubic microns of corpuscles. From values given by many there will be in each milliliter of the liquid of the rumen 100 billion cubic microns of protozoa and 15 billion cubic microns of bacteria. It is not at all impossible that 10 per cent of the volume of the rumen contents at the peak of a digestive cycle consists of bacteria and protozoa.

The point of interest is what this mass of living matter means in terms of ingested food.   Hale, Duncan and Huffman (1940) state that with a ration of 20 pounds of alfalfa hay, 87.3 per cent thereof disappeared as such in the rumen, 84.5 per cent of the protein, 74.4 per cent of the cellulose, and 100 per cent of other carbohydrates.

Pearson and Smith (1943) conclude from their observations of the rumen contents (to which urea was added as soon as they were removed from the rumen and kept at 39°C for two to four hours), that at least 25 per cent of the total protein requirement of a cow yielding 25 pounds of milk per day can be synthesized from urea.   From such observations it is concluded that the total transformation of nitrogen from all sources in the rumen to the protein of microörganisms may well approximate the total protein requirements of the animal.

3. *A balanced biology.*   In such a controlled environment as the rumen provides the biota becomes balanced.   No one unit of the system can get far out of line quantitatively, and hence there is no opportunity for the by-products of any form to accumulate and to reach a level that will be injurious to it or to other forms.   If the continuity of the system is to be assured, this balance must be maintained.

It is very probable that some units of the system may be removed without disturbing its continuity for probably here as elsewhere in the mechanism of the body considerable compensation is provided.   The observations of Becker and Everett (1930) indicate that the protozoa may be removed without serious disturbance of the physiological system of the host.   This would seem to indicate that bacterial tissue is as available as food to the host as is that of protozoa.   However, the time element in all such trials as those of Becker may have been so short that changes which may seem insignificant for the short period may become significant when a much longer period is involved.

It seems safe to conclude that the biota established by slow evolution in any species is attuned to the total system, that of the host and the associated microorganisms, and that man had best go slowly in judging the effects of any induced change in the biota of the digestive tract of any species.

One must think that the microörganisms of the rumen are building a mass of new organic matter from that ingested by the host, and that this is done by the transformation of the molecules without the loss of much energy.   Thus the two molecules of alcohol resulting from the fermentation of glucose have almost as much energy as did the original sugar and the same is true if the glucose is changed to lactic acid.   The quality of the new matter in relation to the nutrition of the host can only be surmised.   It seems probable that it is equal in this respect to that ingested, if not superior thereto.

The vitamin requirements of any higher animal cannot be learned unless such animals can be kept, for a considerable part of the normal life span, free from microörganisms.   Some students of nutrition believe that all the higher animals require the same vitamins and possibly in the same relative proportions.   One can learn the kind and amount of vitamins that the food of any species of higher animal must contain for normal life.   This is predicated on the sup-

position that the methods used in the assay of various foods yield the same values as do the metabolic processes of the species in question. If the animal flourishes on food not containing a full complement of vitamins, one must suppose, on the basis that all animals have the same qualitative if not the same quantitative requirements, that the deficiencies are made up through the elaboration of vitamins in the digestive tract by bacteria. It is not known that any protozoa are able to synthesize vitamins. The needed quantity of many of the accessory substances is so small that one can well imagine the formation of an adequate supply of one or more by the bacteria growing in the digestive tract.

It has been shown, Lardinois *et al.* (1944), that the following members of the B-complex are formed in the rumen of the bovine: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin. It is, of course, also possible that they may be destroyed in the tract. Becker's observation, that sheep used the food more completely in the absence of the protozoa of the rumen than in their presence, may be correct when certain kinds of measuring devices are used, but it seems questionable that it is true for the total physiology of the host over long periods.

4. *A biological unit and its evolution.* One should think of the higher form and the associated biota of its digestive tract as a true physiological unit, and that the evolution of the symbiosis has taken place as has that of the physiology and the agents that carry on the chemical work of the body. If a particular element of the biota becomes of no value as the evolution progresses, it tends to disappear from the system, and if the need arises for a new element it will gradually appear.

In the field of enzymology one speaks of an adaptative enzyme, meaning thereby one that is formed under the stimulus of a substratum that has for the first time been presented to the organism. The organism adapts itself thereby to the new situation. May not the same be true in regard to the flora and fauna of the digestive tract? Indeed, it must have happened and be happening if evolution is to be accepted for all forms that have symbiotic relations; and apparently all animals do have such relations with some of the microörganisms in spite of the fact that a condition of sterility of the digestive tract has been reported for some of the animals of the polar regions. If the latter be true, some research foundation should, without delay, establish a laboratory there for the study of the nutritive requirements of those animals, for under such conditions some facts might be revealed that are out of man's reach working in the regions where such sterility of the tract is unknown.

5. *The cecum.* The discussion has been primarily concerned with the biology of the rumen of the bovine and its significance to this host. Other herbivorous animals have mechanisms that seem to serve much the same purpose as the rumen. Thus the large cecum of the horse provides a chamber in which the ingesta, after they have been acted on by the digestive juices of the upper part of the tract, are then exposed to the prolonged action of bacteria and of protozoa. The ability of the horse to digest cellulose is dependent on this mechanism. The mastication of woody material by the horse is less than that by the bovine because the horse has to chew the dry material, while the bovine chews the same

sort of material after it has been soaked, milled, percolated and fermented for a number of hours. The incompleteness of digestion of fibrous material by the horse and also by the rabbit and elephant is shown in the high cellulose content of their feces.

The large cecum of the rabbit plays a rôle similar to that of the horse. The biology of the ceca of such animals as the horse and the rabbit has not been studied to any extent.

6. *Coprophagy.* The subject of coprophagy is one to which little attention has been devoted in the study of the nutrition of animals. The eating of the feces is claimed to be a constant phenomenon in the rabbit. It is noted in rabbits kept on special diets and has been thought to be a consequence of the inadequacy of the diets which the animals attempt to overcome by consuming their feces. With modern techniques this consumption is supposedly prevented by keeping the animals on screens. Apparently this is less successful in the case of the rabbit than is commonly supposed, since the animal takes a part of the feces as they are voided, a process that will occur whether the animal is on screens or not. Apparently two types of feces occur, the hard type, the one that is evident, and a moist type that is consumed as voided and hence escapes notice. They are termed the day and night types respectively. Recent observations by Southern (1940) indicate that the swallowing of the moist feces is a natural phenomenon, not a reaction to an artificial environment as is so commonly supposed at present. It is interesting to consider how such a phenomenon has escaped notice until recently, especially in view of the domestic raising of rabbits, and of the quite constant presence of the moist type of feces in their stomachs. Such an animal as the rabbit utilizes a regenerative mechanism. Some by-products are returned to the system there to be used in the chemical processes of the body.

Coprophagy is also a natural phenomenon in the case of the normal rat, although here as in the case of the rabbit it is commonly supposed to reflect an abnormal and inadequate diet. It is not certain that this animal, as the rabbit, takes the feces as voided, since few observations have been made. Rats so restrained that feces cannot be reached should be observed. Without such observations the needed vitamin content of the food for this animal cannot be learned with any certainty. Coprophagy is practiced by many other animals, either throughout life or for certain periods thereof. Black (1942) has noted that rats on deficient diets prefer feces of normal rats to their own. He also says that coprophagy is always noted in experimental rats. It seems that the animal thus obtains certain substances not otherwise available to it and that these substances are contained in the feces, hence the attraction of the animal to them. When the final chapter is written on the ability of the animal to select its own food from a mixture, many students of nutrition will be surprised at the incompleteness of their knowledge. The instinct of the unhampered animal as a guide to its needs for food is often very surprising, and it may be that man would be as well off if he allowed his instinct to operate as to follow the advice so freely offered today for his guidance in selecting his diet.

7. *Digestion.* The term "digestion" is used in many ways in present-day

literature on nutrition. The physiologist bases his statements regarding the changes which food undergoes in the digestive tract on his knowledge of the inherent enzymes of the body and, of course, on those which he can separate and study *in vitro*. He is thus thinking of certain processes of degradation by which the food is so changed that it can pass through the wall of the intestine or be prepared for absorption. If some of the agents causing this change are derived from the microörganisms growing in the tract, it seems proper to include these agents as part of the digestive mechanism. Thus if an amylase of microbial or of plant origin functions in the digestive tract, there seems to be no reason why the changes produced thereby should not be included under the term. The synthesis of new material, such as takes place in the rumen for example, is quite a different matter, and it is questionable whether it should be included under the term "digestion" any more than if it went on outside the tract. Is the formation of alcohol or of lactic acid a phase of digestion? It would seem desirable to clearly differentiate between the synthetic processes and those of degradation in speaking of digestion in order that the mind of the reader be not confused. If one accepts the definition of digestion offered by Cannon (1936), namely, that it is the intimate process by which the food eaten is transformed from its natural, complex solid, semi-solid or unsuitable fluid state into a state of being soluble in the juices of the small intestine, then little if any digestion takes place in the rumen; while if one accepts the definition of Morrison (1936) namely, that all the changes which food undergoes within the digestive tract to prepare it for absorption and use in the body are known as digestion, one could consider the change of cellulose to bacterial tissue as it occurs in the rumen as digestion. As has been stated earlier, there is no reason to believe that appreciable absorption occurs through the wall of the rumen, since there are too many agents to consume immediately any food material made available and thus to use it before it can reach the absorbing surface which may be many inches away from the point of formation.

From statements frequently made by students of the nutrition of the bovine as to the use by the animal of the by-products of the microbial destruction of organic matter in the rumen, one would conclude that at certain periods in a digestive cycle, the percentage of soluble matter in the liquid of the rumen would be considerable. The writer (unpublished data) has determined the total soluble matter in the liquid of the rumen at various periods in a digestive cycle. The insoluble matter was removed by filtration through porcelain or by prolonged centrifugation. The values found approximated 1.5 per cent of soluble matter, a value not far different from those given for the soluble matter of the bovine saliva. In the case of the horse and rabbit the cecum seems to play, at least to some degree, the rôle that the rumen plays in the polygastric animals. The food that has not been digested in the stomach and small intestine enters the cecum, there to remain and be acted on by bacteria and protozoa with the building up of a mass of microbial tissue which must be digested and the soluble products absorbed in the large intestine, since the reasons against the absorption of soluble products of microbial action from the cecum are similar to those counter-indicating such absorption from the rumen.

In man, the bacterial content of the digesta reaches its lowest level in the stomach and the upper part of the small intestine, and its highest level in the large intestine. MacNeal (1909) states that about 46 per cent of the nitrogen excreted in the feces of man is contained in the cells of bacteria. Mangold (1929) says that 14.7 to 18.7 per cent of the dry matter of bovine feces consists of bacteria. It has also been shown that the bacterial content of bovine feces increases after the same are voided.

The bacterial content of the small and large intestines increases as lower and lower levels are reached. The usual explanation is that these bacteria are living on the undigested residues of the ingesta. This explanation seems inadequate since the food, especially in the ruminant, has been exposed to a series of active agents that would seem able to accomplish all that any of the bacteria of the lower levels of the tract could accomplish. It seems more probable that the food for such bacteria is the organic matter that is excreted into the lower part of the tract. The fact that there is no known food that will cause much decrease in the volume of the feces in man is indicative of this source of food for the bacteria of the lower bowel. Some years ago the writer observed young men who lived on milk alone for over four months. The only additional food taken was an orange or an apple each day. Theoretically the milk should be completely metabolized and the volume of the feces should be greatly decreased, when it represents almost the entire food consumed over a considerable period. This did not happen. The volume of feces was not markedly decreased from that on a mixed diet.

REFERENCES

BECKER, E. R. 1932 The present status of problems relating to the ciliates of ruminants and *Equidae*. Quart. Rev. Biol. 1, 282–297.

BECKER, E. R., AND EVERETT, R. C. 1930 Comparative growths of normal and infusoria-free lambs. Am. J. Hyg., 11, 362–370.

BECKER, E. R., AND TALBOT, M. 1927 The protozoan fauna of the rumen of American cattle. Iowa State College J. Sci. 1, 345–371.

BECKER, E. R., ET AL. 1930 Experiments on the physiological relationships between the stomach infusoria of ruminants and their hosts. Iowa State College J. Sci. 4, 215–251.

BLACK, SIMON. 1942 Studies on the intestinal synthesis of nutritional factors. Doctoral Dissertation, University of Wisconsin.

BUCHNER, P. 1930 Tier und Pflanze in Symbiose. 2te Auflage, Berlin.

CALKINS, G. N. 1938 Biology of the Protozoa. 2nd Edition, Lea and Febiger, Philadelphia.

CALKINS, G. N., AND SUMMERS, F. M. 1941 Relationships between certain protozoa and other animals. Protozoa in Biological Research, Chap. 19, pp. 890–1008 by Kirby, H. Jr., Columbia Univ. Press, N. Y.

CANNON, W. B. 1932 The Wisdom of the Body. W. W. Norton and Co., N. Y.

CANNON, W. B. 1936 Digestion and Health. W. W. Norton and Co., N. Y.

CLEVELAND, L. R. 1923 Correlation between the food and morphology of termites and the presence of intestinal protozoa. Am. J. Hyg. 3, 444–461.

DUKES, H. H. 1942 The Physiology of Domestic Animals. 5th ed., Comstock Pub. Co., N. Y.

EDEN, A. 1940 Coprophagy in the rabbit. Nature 145, 36–37.

FERBER, K. E., UND WINOGRADOWA-FEDOROWA, T. 1929 Zählung und Teilungsquote der Infusorien im Panzen der Weiderkäuer. Biol. Zentrb. 49, 321–328.

HALE, E. B., DUNCAN, C. W., AND HUFFMAN, C. F. 1940 Rumen digestion in the bovine with some observations on the digestibility of alfalfa hay. J. Dairy Sci., 23, 953–967.

JOHNSON, R. C., HAMILTON, T. S., MITCHELL, H. H., AND ROBINSON, W. B. 1942 The relative efficiency of urea as a protein substitute in the ration of ruminants. J. Animal Sci., 1, 236–245.

JUDAY, C. 1942 Summer standing crop of plants and animals. Trans. Wisconsin Acad. Sci., 34, 103–134.

LARDINOIS, C. C., MILLS, R. C., ELVEHJEM, C. A., AND HART, E. B. 1944 Rumen synthesis of the vitamin B complex as influenced by ration composition. J. Dairy Sci., 27, 579–583.

MANGOLD, E. 1929 Der Verdauung der Wiederkäuer. In: Handbuch der Ernährung und des Stoffwechsels der landwirtschaftlichen Nutztiere als Grundlagen der Futterungslehre, Bd. 2. Springer, Berlin.

MACNEAL, W. J., LATZER, L. L., AND KERR, J. E. 1909 The fecal bacteria of healthy man. J. Infectious Diseases 6, 123–169.

MEAD, S. W., AND GOSS, H. 1935 Ruminant digestion without roughage. J. Dairy Sci. 18, 163–170.

MILLS, R. C., LARDINOIS, C. C., RUPEL, I. W., AND HART, E. B. 1944 Utilization of urea and growth of heifer calves with corn molasses or cane molasses as the only readily available carbohydrate in the ration. J. Dairy Sci. 27, 571–578.

MONROE, C. F., AND PERKINS, A. E. 1939 A study of the pH values of the ingesta of the bovine rumen. J. Dairy Sci. 22, 983–991.

MOON, F. E., AND VARLEY, N. 1942 The utilization of rumen contents as animal fodder. Vet. Record 54, 359–360.

MORRISON, F. B. 1936 Feeds and Feeding. 20th ed., Morrison Pub. Co., Ithaca, N. Y.

MOWRY, H. A., AND BECKER, E. R. 1930 Experiments on the biology of the infusoria inhabiting the rumen of goats. Iowa State College J. Sci., 5, 35–60.

NEVENS, W. B. 1928 Effects of fasting and the methods of preparation of feed upon the digestive processes in dairy cattle. J. Agr. Research, 36, 777–794.

PEARSON, R. M., AND SMITH, J. A. B. 1943 The utilization of urea in the bovine rumen. Biochem. J., 37, 153–164.

RITZMAN, E. G., AND BENEDICT, F. G. 1938 Nutritional Physiology of the Adult Ruminant. Carnegie Inst. Wash. Pub. 494.

SCHALK, A. F., AND AMIDON, R. S. 1928 Physiology of the ruminant stomach (bovine). Bull. 216, No. Dak. Agr. Expt. Sta.

SCHEUNERT, A. 1925 Verdauung der Wirbeltiere. In: OPPENHEIMER, Handbuch der Biochemie des Menschen und der Tiere, 2. Aufl., Bd. V, 56–216. Fischer, Jena.

SCHEUNERT, A., UND SCHIEBLICH, M. 1927 Einfluss der Mikroorganismen auf die Vorgänge im Verdauungstraktus bei Herbivoren. In: Handb. d. normalen und pathol. Physiologie. Bd 3, 967–1000. Springer, Berlin.

SCHWARTZ, W. 1935 Untersuchungen über die Symbiose von Tiere mit Pilzen und Bakterien. Arch. Mikrobiol., 6, 369–460.

SOUTHERN, H. N. 1940 Coprophagy in the wild rabbit. Nature, 145, 262.

TWORT, F. W., AND INGRAM, G. L. Y. 1913 A Monograph on Johne's disease. London.

VOGLER, K. G., AND UMBREIT, W. W. 1941 The necessity for direct contact in sulfur oxidation by Thiobacillus thiooxidans. Soil Sci., 51, 351–337.

THE OUTLINE CLASSIFICATION USED IN THE BERGEY MANUAL OF DETERMINATIVE BACTERIOLOGY

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The following outline classification has been developed from previous classifications by the Editorial Board of the Manual for use in the sixth edition of the Bergey Manual of Determinative Bacteriology. The authors gratefully acknowledge their indebtedness to those who have previously studied this subject, especially those whose outlines are given below. Many suggestions have also been received from the bacteriologists who are currently collaborating in the preparation of the new edition of the Bergey Manual. It is hoped that the new (6th) edition will be ready in 1945.

Historical Setting. In order to permit comparisons to be made readily, some of the outline classifications of the fission fungi developed since 1900 are given here in abbreviated form.

1. The outline used by Chester (1) is based on Migula's outline as given by Engler and Prantl (2).

Class *Schizomycetes.*

    Family A. *Coccaceae*, spherical bacteria.

        B. *Bacteriaceae*, rod-shaped bacteria.

        C. *Chlamydobacteriaceae*, sheathed filaments.

        D. *Beggiatoaceae*, filamentous sulfur bacteria.

        E. *Mycobacteriaceae*, acid-fast rods, true and false branching filaments.

2. Orla-Jensen (3) developed an outline in which he included the purple and sulfur bacteria, and the actinomycetes.

Class *Schizomycetes.*

    Order I. *Cephalotrichinae*, polar flagellate bacteria.

        II. *Peritrichinae*, peritrichous bacteria.

3. Buchanan (4) formulated an even more comprehensive outline as it includes the slime bacteria and the spirochaetes.

Class *Schizomycetes.*

    Order I. *Eubacteriales*, true bacteria.

        II. *Actinomycetales*, diphtheria and tubercle bacillus, actinomyces.

        III. *Chlamydobacteriales*, filamentous, alga-like bacteria.

        IV. *Thiobacteriales*, sulfur bacteria.

        V. *Myxobacteriales*, slime bacteria.

        VI. *Spirochaetales*, spirochaetes.

4. In the preliminary report by Winslow *et al.* (5), emphasis is placed on the true bacteria, the remaining orders that are mentioned being listed by name and by brief description only.

Class *Schizomycetes.*

Order A. *Myxobacteriales*, slime bacteria.

B. *Thiobacteriales*, sulfur bacteria.

C. *Chlamydobacteriales*, filamentous bacteria.

D. *Eubacteriales*, true bacteria.

Supplement: Organisms intermediate between bacteria and protozoa,—*Spirochaetaceae.*

5. The final report by Winslow *et al.* (6), is more comprehensive as it includes the *Actinomycetales.*

Class *Schizomycetes.*

Order A. *Myxobacteriales*, slime bacteria.

B. *Thiobacteriales*, sulfur bacteria.

C. *Chlamydobacteriales*, alga-like bacteria.

D. *Actinomycetales*, as above.

E. *Eubacteriales*, true bacteria.

6. The outline classification as used by Bergey *et al.* (7) in the first four editions of the Manual is identical with that of Buchanan (4).

7. Lehmann and Neumann (8) do not include the purple, sulfur, slime or alga-like bacteria in their outline.

Class *Schizomycetes.*

Order I. *Schizomycetales*, true bacteria.

II. *Actinomycetales*, as above.

8. Kluyver and van Niel (9) include the true bacteria, and all purple and sulfur bacteria but do not list the actinomyces and slime bacteria.

Family A. *Pseudomonadaceae*, polar flagellate bacteria.

B. *Micrococcaceae*, spherical bacteria.

C. *Mycobacteriaceae*, diphtheria and tubercle bacillus.

D. *Bacteriaceae*, true bacteria.

9. Rahn (10) discusses only the groups found among the true bacteria in his outline.

Order I. *Eubacteriales.*

Sub-order A. *Endosporales*, spore-forming rods.

B. *Asporales*, non-spore-forming rods.

10. Bergey, Breed, Murray and Hitchens (11) in the 5th edition of the manual recognize a seventh order *Caulobacteriales* as organized by Henrici and Johnson (12).

Class *Schizomycetes.*

Order I. *Eubacteriales*, true bacteria.

II. *Actinomycetales*, as above.

III. *Chlamydobacteriales*, filamentous bacteria.

IV. *Caulobacteriales*, stalked bacteria.

V. *Thiobacteriales*, sulfur bacteria.

VI. *Myxobacteriales*, slime bacteria.

VII. *Spirochaetales*, spirochaetes.

11. Prévot (13) is primarily interested in anaerobic bacteria. He accepts the outline used in the 5th edition of the Bergey Manual but regards *Schizomycetes* as a kingdom, and the seven orders as classes.

12. Stanier and van Niel (14) have drawn up a comprehensive outline in which they regard the fission algae and fission fungi as comprising a separate kingdom.

Kingdom *Monera*.

    Division I. *Myxophyta*, fission algae.

          II. *Schizomycetaca*, fission fungi.

   Class I. *Eubacteriae*.

      Order   I. *Rhodobacteriales*, purple bacteria.

            II. *Eubacteriales*, true bacteria.

           III. *Actinomycetales*, actinomyces.

   Class II. *Myxobacteriae*.

      Order I. *Myxobacteriales*, slime bacteria.

   Class III. *Sphirochactae*.

      Order I. *Sphirochactales*, spirochaetes.

Appendix: Group I. Filamentous unsheathed organisms.

            Family I. *Leptotrichaceae*.

                 II. *Crenothricaceae*.

        Group II. Unicellular organisms multiplying by transverse fission.

            Family I. *Achromatiaceae*.

        Group III. Unicellular organisms multiplying by longitudinal fission, or budding, or both.

            Family I. *Pasteuriaceae*. .

OUTLINE USED IN THE 6TH EDITION OF THE BERGEY MANUAL

The revised outline that is to be used in the 6th edition of the Bergey Manual (15) now in press is as follows:

Phylum *Schizophyta* (Fission plants).

   Class I. *Schizophyceae* (Fission algae. Blue-green algae).

       II. *Schizomycetes* (Fission fungi).

     Order I. *Eubacteriales* (True bacteria. Rigid cells that are flagellate when they are motile.)

        Sub-order   I. *Eubacteriineae* (Unattached and do not possess photo-synthetic pigments. Includes Family, *Corynebacteriaceae*).

                 II. *Caulobacteriineae* (Sessile and stalked, attached bacteria.)

               III. *Rhodobacteriineae* (Sulfur purple and non-sulfur purple bacteria.)

     Order II. *Actinomycetales* (Branching, non-motile, mycelial threads.)

         III. *Chlamydobacteriales* (Filamentous, alga-like bacteria. App. *Beggiatoaceae*.)

         IV. *Myxobacteriales* (Slime bacteria, creeping motility).

4. BUCHANAN, R. E. 1917 Studies in the nomenclature and classification of the bacteria. II. The primary subdivisions of the *Schizomycetes*. J. Bact., 2, 155–164.

5. WINSLOW, C.-E. A., *et al.* 1917 The families and genera of the bacteria. Preliminary report. J. Bact., 2, 505–566.

6. WINSLOW, C.-E. A., *et al.* 1920 The families and genera of the bacteria. Final report. J. Bact., 5, 191–215.

7. BERGEY, D. H., *et al.* 1923–1934 Bergey's Manual of Determinative Bacteriology. 1st to 4th ed., Williams & Wilkins Company, Baltimore.

8. LEHMANN, K. B., UND NEUMANN, R. O. 1927 Bakteriologische Diagnostik. 7 Aufl., II. Bd. J. F. Lehmanns Verlag, München.

9. KLUYVER, A. J., AND VAN NIEL, C. B. 1936 Prospects for a natural system of classification of bacteria. Centr. Bakt., II, 94, 369–403.

10. RAHN, O. 1937 New principles for the classification of bacteria. Centr. Bakt., II, 96, 273–286.

11. BERGEY, D. H., BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1939 Bergey's Manual of Determinative Bacteriology. 5th ed., Williams & Wilkins Company, Baltimore.

12. HENRICI, A. T., AND JOHNSON, D. E. 1935 Stalked bacteria, a new order of *Schizomycetes*. J. Bact., 29, 3–4 and 30, 61–92.

13. PRÉVOT, A.-R. 1940 Manual de Classification et de Determination des Bactéries Anaérobies. Monographies de l'Institut Pasteur. Masson et Cie., Paris.

14. STANIER, R. Y., AND VAN NIEL, C. B. 1941 The main outlines of bacterial classification. J. Bact., 42, 437–466.

15. BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1944 The outline classification used in the Bergey Manual for Determinative Bacteriology. J. Bact., 47, 421.

16. BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1940 Outline of classification introduced in the fifth edition of the Bergey Manual of Determinative Bacteriology. Centr. Bakt., II, 102, 417–424.

17. VAN NIEL, C. B. 1944 The culture, general physiology, morphology, and classification of the non-sulfur purple and brown bacteria. Bact. Rev., 8, 1–118.

18. ZoBELL, C. E., AND UPHAM, H. C. 1944 A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanography, La Jolla, 5, 253.

19. METCHNIKOFF, É. 1888 *Pasteuria ramosa:* Un représentant des bactéries à division longitudinale. Ann. inst. Pasteur, 2 (4), 165–170.

20. LEHMANN, K. B., UND NEUMANN, R. O. 1907 Bakteriologische Diagnostik. 4 Aufl., Bd. II. J. F. Lehmanns Verlag, München.

21. PRINGSHEIM, E. G. 1923 Zur Kritik der Bakteriensystematik. Lotos, 71, 357–377.

22. BUCHANAN, R. E. 1938 Bacteriology. The Macmillan Company, New York, 4th ed., 48–64.

23. GIESZCZYKIEWICZ, M. 1939 Zur Frage der Bakterien-Systematik. Bull. Intern. acad. polon. sci., Classe sci. math. nat., B I, 9–30.

24. HOLMES, F. O. 1939 Handbook of Phytopathogenic Viruses. Burgess Publishing Company, Minneapolis, Minn.